Characteristics and Use of Okara, the Soybean Residue from Soy Milk Production—A Review

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Large quantities of *okara* produced annually pose a significant disposal problem. It contains mostly crude fiber composed of cellulose, hemicellulose, and lignin, about 25% protein, 10−15% oil, but little starch or simple carbohydrates. It is a suitable dietary additive in biscuits and snacks because it reduces calorie intake and increases dietary fiber. The high-quality protein fraction has good water holding and emulsifying qualities and contains a peptide with anti-hypertension effects. The pectic polysaccharides fraction is suitable for thickening acid milk products. Okara fermented with *Actinomucor elegans* (*meitauza*), *Aspergillus oryzae* (*koji*), *Neurospora intermedia* (*ontjom*), and *Rhizopus oligosporus* (*tempe*), on consumption, reduces cholesterol level and contains substances that counteract dietary free radicals. Unique and useful products produced by *Bacillus subtilis* and *Penicillium simplicissimum* on *okara* include surfactin and iturin A (fungicidal), okaramines A, B, D−F (D is insecticidal), an oleanane triterpene, and two dihydroquinolinones (one toxic for *Artemia salina*). Okara has been used as silkworm food and in the production of ceramics.

Keywords: *Actinomucor elegans; antioxidant activity; Artemia toxicity; Aspergillus oryzae; Bacillus subtilis; cellulose; cholesterol reduction; dihydroquinolinones; food waste; hemicellulose; insecticidal activity; iturin; okaramine; Penicillium simplicissimum; Neurospora intermedia; nutritional supplement; oleanane triterpene; Rhizopus oligosporus; soybean; surfactin; thickening agent*

INTRODUCTION

The residue left from ground soy beans after extraction of the water extractable fraction used to produce soy milk and *tofu*, is called *okara*, draff (Noguchi, 1987), *tofukasu* (Matsumoto and Take, 1980), or *soy pulp*, *dou zha* (Chinese), *bejee* (Korean), and *tempe gembus* (Indonesian) (Liu, 1997). About 1.1 kg of fresh okara is produced from every kilogram of soybeans processed for soy milk (Khare et al., 1995a). Wang and Cavins (1989) found that 30% of bean solids, 20% of bean protein, and 11% of oil ended up in the okara. Huge quantities of okara are produced; e.g. in Japan about 700 000 tons of okara were produced from the tofu production industry in 1986 (Ohno et al., 1993). The okara is sometimes used as an animal food (Noguchi, 1987), in Japan most is burnt as waste (Ohno et al., 1993), and in Hong Kong it is dumped in land fills. Much research has been undertaken to devise uses for this byproduct, some of which has been directed to the development of other processed human foods. Its use as a human food, however, is constrained by its high fiber content.

The production methods of soy milk, from which okara is produced, vary, but the ratio of water to beans is usually between 8:1 to 10:1 (Liu, 1997). In the Chinese method beans are soaked, rinsed, and ground, and the okara is filtered off; in the Japanese method the rehydrated beans are cooked before grinding and filtering (Liu, 1997). These processes should have profound effects on the microbiological quality of the resulting okara. However, a new process, the Illinois method, has been developed in which no okara is produced as the whole bean is homogenized, but the resulting soy milk unfortunately has undesirable organoleptic properties (Liu, 1997).

The only review of okara appears to be that brief review by Liu (1997) in his excellent book on the soybean. The objective of this review is to examine the properties of okara and to review the processes and their outcomes of various studies carried out to develop useful products from this waste product so that the environmental consequences of its disposal can be avoided.

PRESERVATION OF OKARA

The extraction of soy milk involves the separation of the liquid fraction by mechanical means. The remaining okara is wet to slighly damp depending on the efficiency with which the water phase is removed from the draff. The okara putrefies very quickly because it has a high water activity (Noguchi, 1987). Kato et al. (1986) tried to prevent microbiological spoilage of the okara using lactic acid bacteria. Yogurt and dried lactic acid bacteria were added to okara, with or without added 1% glucose, and held under aerobic and facultatively anaerobic conditions. Under aerobic conditions, spoilage occurred quickly but when the okara was packaged in a polyethylene film bag or in a screw-cap bottle, a lactic fermentation occurred so that the pH value was lowered to less than 4.2, which inhibited growth of spoilage bacteria at 37 °C for 4 days or longer. A dried lactic acid bacterial starter, particularly *Lactobacillus plantarum*, was a

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10.1021/jf980754l CCC: $18.00
Published on Web 01/29/1999

Table 1. Percentage Protein, Fat/Oil, Crude Fiber, and Carbohydrates, on a Dry Matter Basis, Reportedly Found in Okara

protein	crude fat/oil	crude fiber	carbohydrates	ref
24.0 (18.2−32.2)	15.2 (6.9−22.2)	14.5 (9.1−18.6)		Bourne et al. (1976)
25.4−28.4	9.3−10.9	52.8−58.1	3.8−5.3	Van der Riet et al. (1989)
26.8 ± 1.0	22.3 ± 1.5			Guermani et al. (1992)
26.8	12.3		52.9	Ma et al. (1996)

better starter than yogurt, and spoilage was best prevented by inoculating okara (1% glucose added) with 10^6 dried lactic acid bacteria/g and incubating in semi-anaerobic conditions at 30−37 °C.

Another method of preservation is to dry the okara soon after production. Muramatsu et al. (1995a−c) studied the dehydration and cohesion properties of the okara using added water absorbing synthetic polymers in various forms. Hirotsuka et al. (1987) of the Fuji Oil Co. Ltd. obtained a U.S. patent for a process of drying okara while preserving its desireable qualities of water binding capacity and whiteness. The okara, with water content adjusted to 65−75 wt %, was fed to a pneumatic conveying drier 50 cm above the pulverizer to which it was allowed to fall by gravity where it was reduced to particles, 1.5−13 mm in size. A nonstick material, such as poly(tetrafluorethylene) or a ceramic, coated the inner surface of the pulverizer which prevented adhesion of the okara to the surface and avoided undesirable browning and caking.

Fujigami et al. (1980) demonstrated that okara of particle diameter <1.4 mm and a moisture content of 0.4 kg water·(kg^{-1} dry matter) at a bed depth of 40 mm could be dried satisfactorily using a vibro-fluidized bed under vacuum. On the other hand Tadano et al. (1981) used a pneumatic drying system to dry okara. The feedstock, containing 84.5% moisture and hot air (208−254 °C) at a low velocity (3.20 m·s^{-1}), was used in a relatively large drying tower. The moisture content of the output varied. The rehydration properties and shelf life of the dried okara containing 25.3% moisture were good but of short duration, 0.5 month at 5 °C, and for that okara containing 7.4% moisture rehydration was poor with a storage life of 8 months at 5 °C (Tadano et al., 1981). It is probable that the higher moisture content okara had a very high water activity, as water activity depends on the concentration of solute particles which should be low in soy milk and consequently in okara. Earlier Chung et al. (1978) stored okara in a dried pellet form. Provided the pellets, 3 mm by 10 mm, formed from okara containing about 38% moisture, were dried within 95 min at 120 °C in an air flow of 160 ft·min^{-1}, there was no color deterioration from the process.

OKARA COMPOSITION

The proximate composition of the okara will depend on the amount of water phase extracted from the ground soybeans and whether further water is added to extract residual extractable components. If moisture is reduced to very low levels, then residual soy milk and its soluble and colloidal components will also be reduced to extremely low levels. On a wet weight basis Tadano et al. (1981) found the proximate composition of okara to be 84.50% moisture, 4.73% protein, 1.50% lipid, 7.00% sugars, 1.50% fiber, and 0.40% ash at pH 6.71. A summary of the proximate compositions found on a dry matter basis in four studies is shown in Table 1. Bourne et al. (1976) reported the proximate composition of soy milk residue, hulls included, from 30 cultivars, with a mean moisture value of 76.8% (range 71.5−79.9%). The crude fiber values reported by Van der Riet et al. (1989) are significantly higher than those reported by Bourne et al. (1976), but the former reported soluble fiber levels of 12.6−14.6%, which are similar to the levels for crude fiber that the latter reported (Table 2), and insoluble fiber levels of 40.2−43.6% on a dry matter basis. The fiber component was reported by Guermani et al. (1992) to be 12.1 ± 1.2% hemicellulose, 5.6 ± 0.9% cellulose, 11.7 ± 1.4% lignin, and 0.16 ± 0.07% phytic acid. The phytic acid value is different from that reported by Van der Reit et al. (1989) (Table 2). The main component of the dietary fiber in okara is ruptured cotyledon cells, and the seed coat does not behave the same way as the cotyledon cells when being macerated by various means (Kugimiya, 1995).

Table 2. Proximate Composition, Mineral Analysis, and Vitamin Analysis on a Dry Matter Basis of Okara Prepared from Three Cultivars of Soybeans (Van de Reit et al., 1989)

Proximate Composition, g/(100 g)

cultivar	protein[a]	oil	carbohydrates	phytic acidin	soluble fiber	soluble fiber	total fiber
Edgar	28.4	9.6	5.3	0.5	42.0	14.6	56.6
Hutton	25.4	10.9	3.8	1.2	43.6	14.5	58.1
Prima	26.2	9.3	4.6	0.9	40.2	12.6	52.8

Minerals and Vitamins, mg/(100 g)

	ash	Ca	Mg	Fe	Na	K	Cu
Edgar	3200	260	163	6.2	16.2	1046	1.1
Hutton	3700	428	158	7.2	19.1	1094	1.1
Prima	3000	286	165	8.2	18.4	1233	1.2

	Zn	Mn	P	thiamin	riboflavin	nicotinic acid
Edgar	3.8	2.5	396	0.59	0.04	1.01
Hutton	3.5	3.1	444	0.49	0.03	0.82
Prima	6.4	2.3	407	0.48	0.03	1.04

[a] Kjeldahl nitrogen × 5.71.

Table 3. Carbohydrate Contents (g/(100 g)), on a Dry Matter Basis, of Okara from Three Cultivars of Soybeans (Van der Riet et al., 1989)

cultivar	monosaccharides (unspecified)	oligosaccharides			starch
		stachyose	raffinose	sucrose	
Edgar	0.7	1.4	0.3	2.3	0.59
Hutton	0.6	0.9	0.3	1.3	0.68
Prima	0.7	0.9	0.4	1.8	0.79

Van der Reit et al. (1989) reported values for components within the carbohydrate fraction and the mineral and vitamin levels in the okara from his three cultivars (Table 2) and the simple carbohydrate fractions (Table 3). The values indicate the paucity of these components in okara and serve to reinforce the role of the more complex fibrous carbohydrates. Nevertheless the concentrations would be sufficient to encourage the growth of carbohydrate utilizing microorganisms.

Nakayama et al. (1997) reported the elemental analysis of okara to be, on a dry matter basis, as follows: C, 46.3; H, 6.99; N, 3.99; S, 0.25; and ash, 3.59%. The levels

(mg·(100 g^{-1} of dry weight) of minerals and vitamins reported (Khare et al., 1995a) in commercial okara from the Indian tofu industry (namely, Ca, 260; Mg, 163; Fe, 6; K, 1046; riboflavin, 0.59; thiamin, 0.04; and niacin, 1.01) bear an uncanny resemblence to those values reported by Van der Reit et al. (1989) for the Edgar cultivar. The ash values reported by Van der Reit et al. (1989) are lower than those reported by Ma et al. (1996) of 4540 mg·(100 g^{-1}). Ikeda and Murakami (1995) reported values for Ca, Mg, P, and Zn of 227, 136, 391, and 2.73 mg·(100 g^{-1}), respectively, which were generally on the low side when compared with Van der Reit et al. (1989).

UTILIZATION AND FRACTIONATION OF OKARA

It would be ideal if okara could be used as a human food; however, some components in it have antinutritional or adverse effects when consumed, at least in large quantities. The phytic acid (inositol hexaphosphate) in okara can reduce Ca balance and reduce the availability of some metal ions (Matsuo, 1996). The soluble carbohydrates include raffinose and stachyose, which cause meteorism (flatulence). However, Liu (1997), reporting work done by Hackler and others in the 1960s, said that the protein in okara is of better quality than that from other soy products; e.g., the protein efficiency ratio of okara was 2.71 but that of soymilk was only 2.11. On the other hand Wang and Cavins (1989) reported that the ratio of essential amino acids to total amino acids was similar to tofu and soy milk.

Because of the physical manner in which soy milk is removed from okara, some soluble (colloidal) protein may remain in the interstices of the particles. This can be rinsed away, leaving a water-insoluble fraction of soy protein, but the protein extracted depends on whether the okara has been heat treated (Yamauchi et al., 1994). In non-heat-treated okara extracted with 0.3 M NaCl, sodium dodecyl sulfate gel electrophoresis under nonreducing conditions showed that the protein consisted of a single 42 kDa band which was the same as basic 7S globulin. However, in heat treated okara this band was not formed due to its insolubility, possibly because of cross-links formed through SS bonds. Pepsin digests of the salt extracted globulin and water extracted soybean protein were fractionated by HPLC into single peptides which were screened for inhibition of angiotensin I-converting enzyme (ACE), which converts angiotensin I to angiotensin II which, in turn, constricts arteries and leads to hypertension and raised blood pressure (Martin, 1994). One peptide from the globulin, whose amino acid sequence was Val-Met-Asp-Lys-Pro-Gln-Gly, and four peptides from the water-soluble digest were ACE inhibitory, having IC_{50} values between 14 and 53 μM (Yamauchi et al., 1994).

Khare et al. (1993) investigated protein isolated from okara for its suitability as a food protein. The in vitro digestibility of the protein was 80%, and it exhibited a water absorption capacity of 8 g·g^{-1} and oil and emulsification capacities of 9 g·g^{-1} and 12 mL·g^{-1}, respectively. Ma et al. (1996) recovered 53% of the protein from okara using a solution at pH 9 at 80 °C for 30 min. The extracted protein was then isolated using isoelectric precipitation at pH 4.5. The amino acid profile of the protein, in which methionine and cysteine were the limiting amino acids, was similar to that of the FAO scoring pattern and with high in vitro protein digestibility. Okara protein isolate was compared with a commercial soy isolate, and protein aggregation was suggested as the reason for lower protein solubility at low and high pH, but emulsifying, water and fat binding, and foaming properties were similar for the two protein sources.

Table 4. Composition of the Fiber Component in Okara from the Cotyledon and Seed Coat, g/(100 g of Okara) (Takahashi, 1968)

component	cotyledon	seed coat
hemicellulose	4.7−5.2	1.1−1.3
cellulose	0.5−0.6	2.7−3.7
hemicellulose galactan	2.4−2.7	0.3−0.38
hemicellulose araban	2.0−2.2	
hemicelluolse pentosan		0.51−0.63

Takahashi (1968), seeking to increase tofu yield, analyzed four samples of soybeans after soaking at 30 °C overnight. The hemicellulose content of okara could be fractionated into hot-water-soluble, normal-soluble, and alkali-soluble hemicellulose at a ratio of 5:19:4. Cellulose levels were higher in the seed coat than in the cotyledon but the converse was true for the hemicellulose (Table 4). Araban was present in the cotyledon only, and pentosan was present in the seed coat only, but galactan, which was at a higher concentration in the cotyledon, was present in both. He concluded that cellulase could not be used to increase the yield of the soybean curd because of the paucity of cellulose in the cotyledon. Hisamatsu et al. (1995) found that arabinogalactan was the main constituent of hemicellulose, the alkaline extract (24% KOH) from okara.

Yamaguchi et al. (1996a) developed a method using sodium hexametaphosphate for extracting from okara almost all the pectic polysaccharide alone with no contaminating protein. The extraction conditions required 50 times the volume of a 2% hexametaphosphate solution at 100 °C for 2 h. The extracted polysaccharide was compared with commercial products on the market and was found to have the same viscosity, molecular weight, and sugar composition, and molecular weights or neutral sugar compositions were no different. However, one difference between the hexamonophosphate and commercial polysaccharides was in the galacturonate distribution in the molecules. Yamaguchi et al. (1996b) investigated this difference and separated the polysaccharide into galacturonate poor and galacturonate rich fractions by (diethylamino)ethyl−cellulose chromatography. The fractions were exhaustively degraded by three kinds of pectinase, *exo*- and *endo*-polygalacturonases, and *exo*-polygalacturonate lyase, and two kinds of hemicellulase, *exo*-galactanase and *exo*-arabinase. The results showed that these polysaccharides are comprised of regions of galacturonan and rhamnogalacturonan which carry side chains mainly composed of homogeneous arabinan and galactan. Both reducing and nonreducing ends of the polysaccharide chains had galacturonan regions.

The hemicellulose has been used by the Fuji Oil Co., to produce, by a U.S. patented process (Maeda et al., 1998), a low-viscosity water-soluble carbohydrate that can be used to stabilize soluble protein under acid conditions, e.g. in acidified milk beverages. In the process the hemicellulose is heated at 40−90 °C at pH 9−14 to remove methyl groups from the carboxyl groups on uronic acid, followed by extraction at a temperature of 80−130 °C with pH adjusted to 3−6. The resulting

carbohydrate is composed of rhamnose, fucose, arabinose, xylose, galactose, glucose, and uronic acid.

FERMENTED PRODUCTS FOR HUMAN CONSUMPTION

Matsumoto and Take (1980) examined the possibility of producing a *natto*-like product from okara and tested 15 strains of *Bacillus natto* isolated from commercial *natto*. They examined the biochemical characteristics of the bacteria which included growth on glucose bouillon medium, their production of vitamins such as thiamin, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, folate, cyanocobalamine, and biotin, and the organoleptic qualities of the *natto* produced. Five strains were further tested for growth rate, amylase activity, and the organoleptic qualities of the *natto* so that finally one strain was selected for the purpose.

The traditional Chinese product called meitauza has been produced with okara using *Actinomucor elegans* in a solid-state fermentation (Kronenberg and Hang, 1984). During an 80 h fermentation at 15 °C an acid protease was produced; there was significant liberation of ammonia and an 8-fold increase in nonprotein nitrogen, accompanied by a rise in pH from 5.48 to 7.50. This product has also been used as a model for studying solid-state fermentations (Kronenberg and Hang, 1985). An Instron universal testing machine and a Chatillon hand operated force gauge were used to estimate mycelial binding by measuring the force needed to penetrate meitauza. Their studies showed that mycelial growth and not water loss or crust formation caused increased firmness during fermentation. The force measurements could be used to monitor the degree of mycelial growth during solid-state growth.

Okara was fermented using the tempe fungus, *Rhizopus oligosporus* (*R. oligosporus*) and the koji fungus *Aspergillus oryzae* (*A. oryzae*) (Matsuo, 1989a,b), to improve its nutritional qualities as a high-fiber, low-energy foodstuff suitable for human food. The *R. oligosporus* cells, by multiplication, formed a network in cavities within "soybean cells", and *A. oryzae* grew around the inner cell wall of the soybean. Both fungi improved, on a dry matter basis, protein digestibility (80 to 84%), and the nutrients in the resulting okara were increased as it contained more free amino acids (0.02 to 0.41%), acid-soluble N (0.15 to 0.84%), free sugars (12 to 18%) and inorganic P, and less fiber (56.6 to 49.5%) than did the controls. The dietary fiber content of processed okara was greater than 50%, but its energy content was only half that of wheat flour (Matsuo, 1989a). In addition, also increased were the water-holding and emulsifying capacities, oil-absorbability (9.2 to 41.5 g·(g^{-1} of dry weight)) and antioxidant activities of okara fermented with both fungi. Matsuo (1997b) also prepared Indonesian *ontjom* (*oncom*) from okara using *Neurospora intermedia* to give an orange colored product. After incubation for up to 4 days the components in the original okara changed (protein content, 22% to 27%; fat content, 15% to 9%), and the insoluble fiber content decreased. This suggests that the fungus broke down some of the insoluble fiber. The resulting product had good organoleptic properties: it contained no soy bean flavor, it had a smooth mouth feel, and the texture of fried *ontjom* resembled chicken.

Matsuo (1990) used *R. oligosporus* to ferment okara without and with rice bran (10% level) to prepare okara tempe. With the added rice bran, the quantity of fungal growth, protease activity, and thiamine and riboflavin contents increased but cellulase and phytase activities and the quantity of inorganic phosphate decreased. Further, the emulsifying capacity and taste of the product were improved by the added rice bran.

NUTRITIONAL CONSEQUENCES OF FERMENTED OKARA FOR MAMMALS

Matsuo (1991) compared the suitability of okara tempe and wheat bran tempe for foodstuff in in vitro tests involving fat digestion and bile acid metabolism in vitro. The okara tempe bound less lipase, oleic acid, and cholic acid than wheat bran tempe, but the neutral detergent fiber of okara tempe had a stronger binding capacity for deoxycholic acid than cellulose and, when fermented by rat cecal flora, had no effect on cholic acid metabolism. These characteristics made the product a suitable foodstuff. Takahashi et al. (1992) also looked at the influence of dietary fiber on the rat gastrointestinal tract. They compared a dietary fiber preparation from soybean with okara and wheat bran, all fed at a level of 5% in the diet. Although none of the fiber sources had an impact on the apparent digestabilities of starch, protein, or fat, the apparent degradabilities of the dietary fiber from soybean, okara, and wheat bran were different, being 83.5, 62.8, and 30.3%, respectively. These differences also showed up in the pH of the cecal contents of the animals with high degradation being associated with lower pH, and thus more acid, acetate and butyrate, being produced in the caecum. The dietary fiber also reduced the transit time in the intestine. They concluded that the dietary fiber, particularly that from the soybean (okara was not mentioned), may be effective in modifying colonic conditions in a positive way.

The proximate compositions of natural okara and fermented okara (*R. oligosporus*) were determined and nutritionally compared (Guermani et al., 1992). Fermentation induced a significant decrease in lipid and lignin contents of okara and, being rich in hemicellulose with a low lignin and phytic acid content, it caused rats in feeding experiments to show lower weight gains and a significant decrease in the protein efficiency ratio while having no influence on food intake. It was a good source of dietary fiber and suitable as another ingredient for the manufacture of low-energy foods.

Matsuo and Hitomi (1992) found lower plasma- and liver-cholesterol levels and triglyceride levels in rats fed a diet containing 50% okara protein enriched with *A. oryzae* (okara koji) (compared with a casein based control diet). The comparison was obtained after feeding for 13 or 3 days following a 2 day starvation period. They also looked at the fiber effects and compared a diet containing 20% soy protein and 20% okara koji, using cellulose as a control, and found that plasma cholesterol levels rose slightly after a 33 day feeding period. They concluded that the protein and fiber components in okara koji made with *A. oryzae* affected lipid metabolism in rats. They found (Matuso and Hitomi, 1993) similar results in a further experiment with okara tempe made with *R. oligosporus*. Plasma levels of cholesterol and bile acid were significantly lower in okara tempe fed animals when compared with a casein fed control group. Levels of cholesterol and bile acid excreted in feces were larger in okara tempe (as a dietary fiber source) fed animals, but there was no difference in plasma levels for these two components in okara tempe or unfermented okara

fed animals, but in the former group of animals liver cholesterol levels were lower. They suggested that there was a complementary action between the water-soluble dietary fiber and the protein levels which lead to a lowering of the plasma cholesterol levels.

Matsuo (1996) also looked at sterol secretion, flatulence, and iron absorption by rats fed either okara fermented with *A. oryzae* or unfermented okara as the dietary fiber source. The sterol excreted in the feces of rats fed with fermented okara was greater than in the feces of rats fed with unfermented okara. The residual insoluble dietary fiber in the feces showed more porosity in the fermented okara than the unfermented okara, which suggested it could, along with the hemicellulose, account for the lowered cholesterol level. Lower levels of raffinose and stachyose were present in the fermented okara diet, so it was suggested they should not cause meteorism. Greater iron absorption by rats on the fermented okara diet suggested a reduced effect of phytic acid on its absorption when compared with unfermented okara.

Fermented okara apparently contains an increased amount of an antioxidant. Yokota et al. (1996a) extracted a crude antioxidant preparation called NTX from fermented okara and compared its action against the pathogenesis of gastric ulcer in stressed rats with α-tocopherol. Factors in the gastric mucosa, such as the areas of gastric mucosal lesions, levels of thiobarbituric acid reactive substances, prostaglandin E_2, and hyaluronic acid, were compared. The work revealed that NTX exerted an antiinflammatory effect on gastric injury in the gastric mucosa. In further work they showed (Yokota et al., 1996b) that in vitro NTX scavenged the superoxide anion which they confirmed also occurred in vivo. They induced foot pad oedema in rats by subcutaneous injection of croton oil. In the NTX fed group the edema was repressed, but groups fed a control diet or vitamin E did not show repressed edema. The repressed edema was associated with lower thiobarbituric acid reactive substances and higher prostaglandin E_2 levels in the tissue. Matsuo (1997a), in further experiments, found that the antioxidants in okara fermented with *A. oryzae* were γ- and δ-tocopherol, the isoflavones genistin, daidzein, genistein, and 3-hydroxyanthranilic acid. An 80% methanol extractant of the fermented okara showed stronger inhibition of linoleate peroxidation than hexane or hot water extractants and showed accelerated formation of 12-hydroxy-eicosatetraenoic acid in membrane lipids in vitro. In vivo effects were tested by feeding rats on a vitamin E deficient diet with fermented okara or okara supplemented with oxidized oil. In rats fed the oxidized oil supplemented okara the body weight gain, the thiobarbituric acid value in the plasma, and glutathione peroxidase activities in plasma and liver were lower than in rats fed the fermented okara. This suggested that the antioxidants in the fermented okara scavenged lipid peroxides in vivo.

Okara alone has some antinutritional qualities; however, fermented okara may have definite advantages in a diet. It can act as a suitable replacement for digestible food in a prepared food to reduce calorie intake, it can reduce cholesterol levels in the blood stream, and as a food that contains antioxidant activity, similar to vitamin E, it can can reduce the level of free radicals in the body.

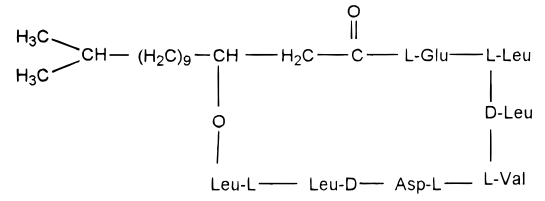
FERMENTATION FOR NONFOOD PRODUCTS

The high-temperature treatment used in the Japanese method of production should reduce the microbial load in okara to very low levels and if used very soon after separation should be ideal for use as a base for a controlled microbial fermentation. Okara from the Chinese method of production would present a different microbiological picture. A number of studies have involved the use of okara as a base for fermentations.

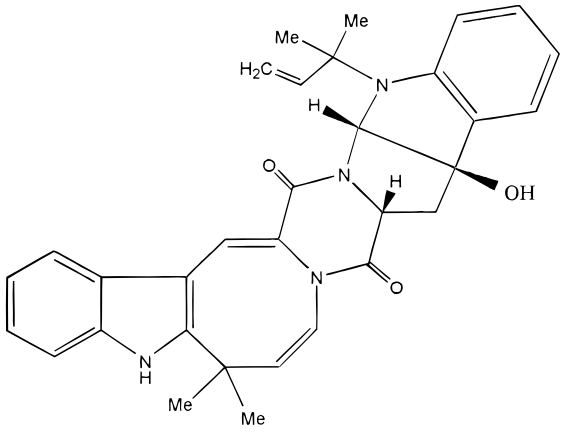
Okara was used as a base for growing *Bacillus subtilis* NB22 to produce iturin A, a cyclic heptapeptide of α-amino acids connected by long β-amino acids, which is a fungicide effective against serious plant pathogens (Ohno et al., 1996). In their studies they used the okara in a solid-state fermentation in 15 g and 3 kg fermentation sizes and produced up to 2 g iturin A·(kg^{-1} of wet weight okara) (11 g·(kg^{-1} of dry weight okara)). Maximum production occurred at 82% moisture and 25 °C. The 3 kg production system was conducted in an 8 L glass jar, and during the fermentation at 25 °C the temperature at the center reached about 45 °C.

Earlier Ohno et al. (1995a) studied the effect of incubation temperature on the concurrent production of iturin A and surfactin by *B. subtilis* RB14, a wild-type surfactin producer (Ohno et al., 1995b), in a solid-state fermentation on okara. Surfactin inhibits fibrin clotting and lyses erythrocytes, sphaeroplasts, and protoplasts and is one of the most powerful biosurfactants known, being capable of lowering the surface tension of water from 72 to 27 $mN \cdot m^{-1}$ (Ohno et al., 1995b). It is a lipopeptide consisting of *iso*-C_{15}-hydroxy carboxylic acid and a seven member ring structure of amino acids (Figure 1): L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Leu (Nakayama et al., 1997), or 3-hydroxy-13-methyl-tetradecanoic acid amidated to the N-terminal of the heptapeptide (Georgiou et al., 1992). The best temperature for iturin A production was 25 °C, and at 42 °C iturin production ceased; but the best temperature for surfactin was 37−42 °C (Ohno et al., 1995a). The gene responsible for surfactin production was transferred to another *B. subtilis* strain (MI113) and grown in a solid-state fermentation on okara, and the best temperature and water content for surfactin production determined were as before and 82%, respectively (Ohno et al., 1995b). Surfactin production was as high as 2.0 g·(kg^{-1} of wet weight), which was eight times as high as that of the original *B. subtilis* RB14 at the optimal temperature for surfactin production, 37 °C. Nakayama et al. (1997) produced new surfactin variants by cloning a recombinant *B. subtilis* from RB14C, named MI113 (pC115), which carried a gene for the production of surfactin and iturin A. The new surfactin variants were also produced, along with those previously reported, during solid-state fermentation on okara.

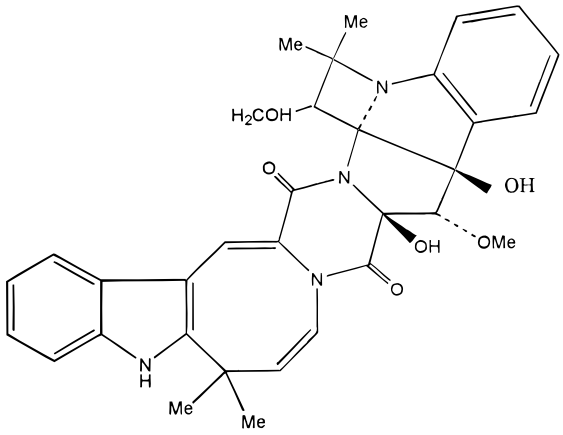
A *B. subtilis* strain when grown on soybean during the production of *natto* produced a fibrinolytic enzyme, but when grown on okara, the activity of the fibrinolytic enzyme was 2.5-fold that formed on the whole soybean after 24−30 h incubation. The fibrinolytic activity was unaffected by the presence of soy milk (1% or 10%) or glucose (1%), but it was affected by the temperature of incubation as fibrinolytic activity at 37 °C was twice that at 30 and 45 °C (Miyamura et al., 1998). This difference between okara and whole soybeans may be due to size of the particle since, when okara particles less than 30



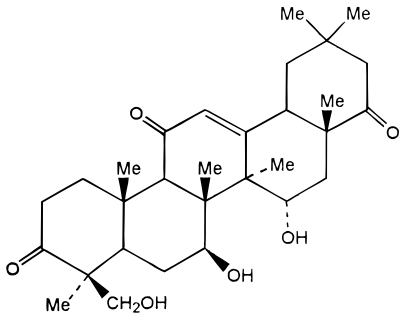
Surfactin



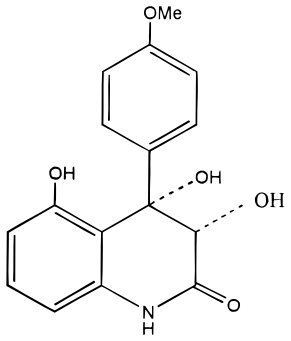
Okaramine A

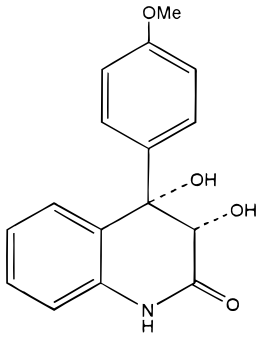


Okaramine D



Oleanane triterpene





Dihydroxyquinolinones

Figure 1. Chemical structures of surfactin, okaramines A and D (produced by *Penicillium simplicissimum* AK-40 and ATCC 90288, respectively), an oleanane triterpene (produced by *Penicillium simplicissimum* ATCC 90288), and two dihydroxyquinolinones (produced by a *Penicillium* sp., NTC-47), produced by microorganisms growing on okara.

mesh (600 μm) were used, the fibrinolytic activity was greater than that found with normal sized okara particles.

A solid-state fermentation of okara with *A. terreus* NCIM 653 and *A. niger* NRRL 330 was used by Khare et al. (1995a) for the production of citric acid. *A. terreus* was used to saccharify the okara and the noncellulolytic *A. niger* used to produce citric acid. The okara was inoculated with a 5% (w/v) inoculum of the fungal cultures and supplemented with 0.1% $(NH_4)_2SO_4$ at pH 8.3 and incubated at 30 °C. Khare et al. (1995a) compared various nitrogen sources and reported the organic sources such as yeast extract and peptone to be inferior to inorganic sources of which $(NH_4)_2SO_4$ was the best. Maximum citric acid yields, 5.1 (g of citric acid)·(100 g^{-1} of dry solids), were obtained when *A. niger* and *A. terreus* were both present throughout the entire cultivation period of 11 days. *A. niger* alone produced only about 20% of the maximum yield of citric acid.

Kitamura et al. (1994) tried using a continuously stirred tank reactor with a working volume of 1 L to study the conditions necessary to saccharify okara with a 1% cellulase solution. In their reactor they determined the best conditions to be a suspension level of the okara of 350 g·L^{-1} at a pH of 4 and a temperature of 40 °C. As the reaction progressed (Kitamura et al., 1996a), there was an increase in reducing sugar, which was accompanied by a rapid decrease in the saccharification

rate, and the solid degradation decreased. These changes could be explained by a Michaelis−Menten equation for competitive inhibition. Kitamura et al. (1996b) then conducted a simultaneous saccharification and fermentation process using baker's yeast to produce ethanol. Although baker's yeast produced ethanol from glucose under the conditions of saccharification, the yield of ethanol from okara under the conditions showed that some reducing sugars produced from okara could not be used by the yeast.

Penicillium simplicissimum AK-40, isolated from soil, was grown on okara in which it produced two novel compounds which exhibited insecticidal properties (Hayashi et al., 1989). The metabolites, named okaramine A ($C_{32}H_{32}N_4O_3$) and B ($C_{33}H_{34}N_4O_5$) (Figure 1), were crystalline, and when the former was fed at 3 $\mu g\cdot(g^{-1}$ of diet), it was insecticidal for the third instar larvae of silkworm but the latter compound was even more insecticidal as 0.1 $\mu g\cdot(g^{-1}$ of diet) was effective. Hayashi et al. (1995) in further studies using *P. simplicissimum* ATCC 90288 found three more okaramine congeners named okaramines D−F, but only okaramine D (Figure 1) showed insecticidal activity. A wide variety of growth media were tested for the production of okaramines A and B, but okara proved to be a superior growth medium for their production (Hayashi et al., 1991), maybe because of the peculiar close juxtposition of fibrous carbohydrate and protein found in okara. Subsequently Hayashi et al. (1996) reported the discovery of a new oleanane triterpene (Figure 1), 7-β,15-α,24-trihydroxy-olean-12-en-3,11,22-trione in okara fermented with the same organism. The usefulness or significance of this new substance has yet to be established. He continued similar studies (Hayashi et al., 1997) with a soil isolate, *Penicillium* sp. NTC47, and isolated two new dihydroquinolinones from fermented okara. The metabolites produced (Figure 1), 3-methoxy-4,5-dihydroxy-4-(4′-methoxyphenyl)quinolinone ($C_{17}H_{17}NO_5$) and 5-deoxy-3-methoxy-4,5-dihydroxy-4-(4′-methoxyphenyl)quinolinone ($C_{17}H_{17}NO_4$), differed in that the former exhibited toxicity for the classic indicator *Artemia salina* at an LC_{50} value of 20 $\mu g\cdot mL^{-1}$, but the latter exhibited no activity at a dose of 100 $\mu g\cdot mL^{-1}$.

NONFERMENTED PRODUCTS

Nolan (1983) reported that the Haarman and Reimer Corp. in the USA developed an okara snack bar. Noguchi (1987), of the Japanese National Food Research Institute, Ministry of Agriculture, Forestry & Fisheries, patented a process in Japan and the USA whereby okara was converted to a textured soybean product. In a single machine the okara was cooked at 160−180 °C and extruded under pressure of 20−200 $kg\cdot cm^{-2}$ at 40−250 rpm, and then pushed through a constricted exit port into a cooling die at 0 °C from which it finally exited. Production of a low-calorie foodstuff based on dietary fiber particles of either less than 5 mm or not greater than 80 mm sourced from okara are covered by at least one U.S. patent (Watanabe et al., 1997) held by Ajinomoto Co., Inc.

Khare et al. (1995b) fortified biscuits with okara at levels from 20 to 100%. They found in taste trials that biscuits with a supplement of 60% okara were most acceptable to people. The protein and dietary fiber contents of these biscuits were 8.72 and 5.98%, respectively. Microbiologically the biscuits stored well for at least 1 month. Kobayashi (1997) obtained a Japanese patent for a process to produce shortening coated okara to be used as a wheat substitute in the making of cakes of good texture and taste.

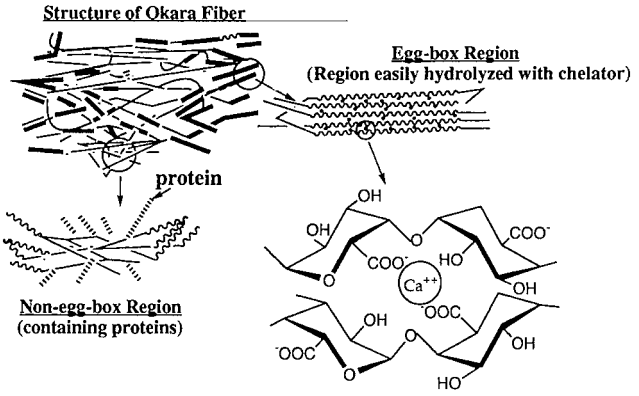


Figure 2. Structural model of the fiber component found in okara. The "egg-box" region contains cross-linked galacturonic acid with Ca^{2+}, and the other region contains protein. Reprinted with permission from Yoshii et al. (1996). Copyright 1996 Japan Publications.

The water-soluble polysaccharides from okara, which can be extracted from okara by hydrolysis, could have potential as food emulsifiers, so the kinetics of okara hydrolysis and the properties of the extracted water-soluble polysaccharides were investigated (Yoshii et al., 1996). Okara was hydrolyzed in an autoclave at pH 4.5 in two volumes of water with or without a chelator (hexametaphosphate or EDTA). Water-soluble polysaccharides with a molecular weight of $\geq 10^5$ were produced by okara hydrolysis, and their emulsification properties were influenced by the amount of protein bound to the polysaccharides. Results also showed that okara consisted of cross-linked galacturonate polymers with Ca^{2+} between carboxylic groups, called "egg-box" regions and non-egg-box regions with hydrophobic proteins (Figure 2). In the absence of a chelator, okara hydrolysis proceeded by a surface degradation mechanism. From an analysis of the water-soluble polysaccharides produced, it seems that the egg-box regions in okara were susceptible to degradation in the presence of a chelator, because the chelator removed Ca^{2+} and loosened the structure of okara.

ANIMAL FOOD

To reduce costs and to even out production through the year, okara was used as a base for a low-cost artificial food for silkworms during the first to the third larval instar stages. Larval growth was good and without disease or reproductive problems (Sumida et al., 1995).

OTHER PRODUCTS

Okara, as described in a Japanese Patent (Hasegawa, 1998), has also been used to make a reinforced ceramic using the pozzolanic reaction. Fly ash (50−70%), clays (10−30%), and okara (10−20%) along with $Ca(OH)_2$ (10−30%) were mixed and heated in an inert gas to 1300−1500 °C. The okara carbonized and then reacted with SiO_2 to form SiC which strengthened the ceramic product.

CONCLUSION

If the production of soymilk and soymilk derivatives continues as the production figures seem to indicate (Liu, 1997), then the utilization and disposal of okara

could well become an increasing problem. The work published shows that it can be used as a food ingredient to boost dietary fiber levels in an otherwise low-fiber diet and that it can be a nutritionally sound addition to the diet in moderate quantities, particularly if fermented with koji and tempe fungi before addition. The huge quantities produced make it an ideal feedstock for fermentations, particularly as mold fermentation of soybeans and other grains has been a tradition in Asia for millenia. One possible use of okara, not mentioned in the literature (O'Toole, 1997), is to add a portion either to the mixture prepared to make koji for soy sauce production or to add a portion to the moromi during soy sauce production.

The discovery of novel chemical substances in okara on which fungi and bacteria have grown is symbolic of a possible application of this product for useful purposes. However, it is not clear whether okara is an essential ingredient for the production of these compounds or whether they would be produced in other laboratory media. In the case of the okaramines it certainly seems that the okara is important for their production, due possibly to the peculiar close mixture of complex carbohydrate and protein, as shown by Yoshii et al. (1996). Certainly the basic structure of the nonprotein and nonoil components of the okara are complex and not straightforward as, for example, starch is. Therefore these complex structures may well be required by the bacteria and fungi to produce these new chemical compounds.

There have been no studies on the use of additives to improve or enhance microbial growth on okara except those by Khare et al. (1995a) who found that $(NH_4)_2SO_4$ was best for citric acid production. Further work on nitrogen sources, and maybe mineral sources (O'Toole, 1997) could yield useful returns.

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Received for review July 8, 1998. Revised manuscript received November 18, 1998. Accepted November 23, 1998.

JF980754L