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*Full Length Research Paper*

# Improvement of nutritional quality and antioxidant activities of yeast fermented soybean curd residue

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**This study evaluated the potential use and improves the health beneficial properties of the soybean waste manufacturing products by solid-state fermentation of six GRAS different yeast strains, including extractable antioxidant activities and bioavailable nutritional compositions. In comparison with non-fermented okara (control), some levels of value addition occurred as a result of the fermentation. The protein contents increased by 20.10- 54.40%, while the crude fiber decreased by 7.38-45.50% with different strains. With all the organisms used, the ash content increased while the carbohydrate and lipid contents were reduced. Total phenolic content and all parameters of antioxidative activities were increased in fermented substrate. The highest significant levels of antioxidant activities were achieved with *Kluyveromyces marxianus* NRRL Y-8281. Results showed that the nutritional quality and antioxidant activities of the substrate were enhanced by solid yeast treatment fermentation. Thus, scope exists for microbial upgrading of this low-quality waste and development of healthy animal feed supplements.**

**Key words:** Solid state fermentation, yeast, waste soybean manufacturing products, antioxidant, protein, fiber.

## INTRODUCTION

Free radicals generated by exogenous chemicals or endogenous metabolic processes in food systems may cause oxidative damage by oxidizing biomolecules and result in cell death and tissue damage (Kehrer, 1993). Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherol and glutathione (Niki et al., 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging (Yang et al., 2000). For animals, pathological stress may be found when they are fed in high stress condition or exposed to some chemicals (Wongputtisin et al., 2007). However, antioxidant supplements or foods containing antioxidants may be used to help the human body and animals to reduce the oxidative damage (Yang et al., 2000).

Synthetic antioxidants are widely used because they are effective and cheaper than natural types. However, the safety and toxicity of synthetic antioxidants have important concerns (Imaida et al., 1983). Much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals (Yang et al., 2000).

Phenolic compounds are plant-derived antioxidants that possess metal-chelating capabilities and radical-scavenging properties (Bors and Saran, 1987; Lopes et al., 1999). Soybean and soybean products, containing various amounts of phenolic compounds have been shown to possess antioxidative ability.

Fermented foods represent on average one-third of total food consumption (Nout and Kiers, 2005) especially fermented soybean that are widespread found in many part of the world as a local food, for example, Tua-nao (Thailand), Natto (Japan), Tempe (Indonesia) and Kinema (India). Those products have been reported on

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**Abbreviations: SSF,** Solid-state fermentation; **DPPH,**1,1-diphenyl-2-picrylhydrazyl.

their higher antioxidant activity via microbial fermentation (Yang et al., 2000; Nout and Kiers, 2005). There are many reports about diversity of microorganisms found in fermented foods, especially in fermented soybean and related products for example *Bacillus* sp., lactic acid bacteria, *Rhizopus oligosporus*, *Aspergillus* sp., *Mucor* sp., *Actinomucor* sp. and yeasts (Hesseltine, 1998; Yang et al., 2000; Lin et al., 2006; Wongputtisin et al., 2007; Zhu et al., 2008).

Okara, a byproduct of tofu, soymilk or soy protein manufacturing is just treated as industrial waste with little market value because of its short shelf life (O'Toole, 1999). In fact, okara generally contains protein up to 25.4-28.4% (dry basis) with high nutritive quality and superior protein efficiency ratio, suggesting that it is a potential source of low cost vegetable protein for human consumption (O'Toole, 1999; Kasai et al., 2004). Several studies have recently investigated the use of the okara, including improving the functional properties of okara protein by acid deamidation (Chan and Ma, 1999), hydrolyzing the okara to increase the digestibility with various enzymes (Kasai et al., 2004); using okara as nitrogen or carbon sources for the solid-state fermentation (SSF) of a microorganism (Hsieh and Yang, 2004; Rashad et al., 2010).

Solid-state fermentation, however, offers the advantages of being more cost- and energy-effective and more environmentally friendly (Krishna, 2005). In addition, it requires minimal post-reaction processing to recover products (Krishna, 2005). Solid-state fermentation process is an alternative way to improve the phenolic content and antioxidant potential in fermented foods. Chemical composition and bioactivity of stale rice were improved by SSF with *Cordyceps sinensis* (Zhang et al., 2008). Lateef et al. (2008) showed that the nutritional qualities and antioxidant activities of different agro- solid wastes were enhanced by SSF. Enrichment of phenolic compound through SSF was reported in black bean (Lee et al., 2008), soybean (McCue and Shetty, 2003; Lin et al., 2006), cranberry pomace (Vattem and Shetty, 2002), fava bean (Randhir et al., 2004) and wheat (Bhanja et al., 2009).

The objectives of this study were to improve the antioxidant level and some nutritional quality of low cost substrate (soybean waste manufacturing products) by solid state fermentation of different yeast strains.

## MATERIALS AND METHODS

### Microorganisms

The following yeasts were used in this study: *Candida albicans* NRRL Y-12; *Candida guilliermondii* NRRL Y-2075; *Kluyveromyces marxianus* NRRL Y-7571 and NRRL Y-8281; *Pichia pinus* and *Saccharomyces cerevisiae* NRRL Y-12632. They were obtained from Agricultural Research Service, Peoria, Illinois, USA. All strains were maintained on yeast malt agar (Wickerman, 1951), then stored at 4°C and sub-cultured monthly. Inoculum was developed by transferring a loopful of each stock culture into a sterile yeast malt medium (Wickerman, 1951). The flasks were incubated at 30 °C on a shaker at 200 rpm for 24 h.

### Materials and reagents

Soybean waste (a by-product from the manufacture of soybean products and it is commonly called okara or soybean curd waste) was obtained from Food Technology Research Institute, Soybean Processing Center, Agricultural Research Center, Giza, Egypt. It was collected freshly and freezed till used.

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemika, ascorbic acid, α-tocopherol, Folin–Ciocalteu reagent, gallic acid, ferrozine, β-carotene and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other reagents were of analytical grade.

### Fermentation of okara

Fresh okara was cooked in an autoclave (121°C, 15 min) and then cooled. Solid state fermentation was performed by evenly spraying 1.0 ml spore suspension (with concentration of ca. $10^8$/ml) into the sterilized okara substrate (50 g). After mixing thoroughly, the inoculated okara substrates were then incubated statically for 3 days at 30°C. During the cultivation period, the okara was stirred and mixed under sterilized conditions after 17 and 25 h of cultivation to accelerate the release of fermentation heat (Lin et al., 2006). The non-fermented okara was prepared without the addition of spore suspension.

### Harvesting

At the end of fermentation, the fermented and non-fermented sterilized okara were oven dried at 60°C, ground and then used for analysis.

### Nutritional composition

Ash, fat and crude fiber contents of both fermented and non-fermented okara substrates were determined following the methods of A.O.A.C. (1980), while the crude protein contents were determined using micro-Kjeldahl method (Nx 6.25). The carbohydrate content was determined by Dubois et al. (1956).

### Antioxidant activities

### Methanolic extracts

The fermented and non-fermented okara substrates were extracted with methanol (1:5 w/v) at 55°C for 2 h in a shaking water bath at 100 rpm. After filtering through Whatman No. 1 filter paper, the extracts were concentrated (Lateef et al., 2008).

### DPPH radical-scavenging assay

The modified methods of Shimada et al. (1992) and Mensor et al. (2001) were used to study the free radical-scavenging activities of the extracts using DPPH. Two ml of methanolic extract or standard (ascorbic acid and α-tocopherol) at a concentration of 2 mg/ml was added to 1.0 ml methanolic solution of 0.3 mM DPPH. The mixture was shaken and left in a dark box to stand for 30 min at room temperature (30 ± 1°C). The blank of each sample was prepared

with 2.0 ml of sample solution with 1.0 ml of methanol instead of DPPH, while 1.0 ml of methanolic DPPH plus 2.0 ml of methanol served as the control. The absorbance of the resulting solution was measured at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation

Scavenging activity (%) = [1 - (absorbance sample/absorbance control)] x 100%.

### *β-carotene/linoleic* system assay

Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated by a modification of the method described by Juntachote and Berghofer (2005). The β-carotene (2 mg) was dissolved in 20 ml of chloroform. An aliquot (3 ml) of the solution was mixed with 40 mg linoleic acid and 400 mg Tween 20 in a 150 ml beaker. Chloroform was removed, and then 100 ml of oxygenated deionized water was added into the β-carotene emulsion and vigorously mixed until completely homogenized. An aliquot (3 ml) of this β-carotene emulsion and 0.1 ml of sample were placed in a capped culture tube and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50°C. Absorbance was measured at 470 nm at interval time (0, 10, 20, 30, 40 min). A control was prepared by using 0.1 ml of distilled water instead of the sample.

Degradation rate of the sample was calculated according to the first order kinetics using Equation (1):

Ln (a/b) x 1/*t* = sample degradation rate

Where, Ln is the natural log; a is the initial absorbance at time zero; b is the absorbance at time 40 min (time of discoloration) and t is the time (min).

The antioxidant activity (AA) was expressed as % inhibition relative to the control using Equation (2):

AA (%) = 100 x (Degradation rate of control - Degradation rate of sample)/Degradation rate of control.

### Reducing power

The reducing activity of the samples was determined essentially following the method of Oyaizu (1986). An equal volume (0.3 ml) of sample, 1.0% potassium ferricyanide and 0.20 M sodium phosphate buffer were mixed thoroughly. The mixture was incubated at 50°C for 20 min and then 0.3 ml of 10% trichloroacetic acid was added. The mixture was centrifuged (6000 rpm) at 4°C for 10 min. The upper layer (0.6 ml) was mixed with 0.12 ml of 0.1% ferric chloride and deionized water (0.6 ml). After 10 min of mixing, the absorbance of this mixture was measured at 700 nm. A higher absorbance of this mixture indicates a higher reducing activity.

### Chelating effects on ferrous ions

$Fe^{2+}$-chelating ability of each extract was determined according to the method of Decker and Welch (1990). The $Fe^{2+}$ level was monitored by measuring the formation of the ferrous ion-ferrozine complex. 1 ml of each methanolic extract was mixed with 3.7 ml methanol, 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine and the mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance indicates a stronger $Fe^{2+}$-chelating ability. The ability to chelate the ferrous ion was calculated as follows:

Chelating effect (%) = [1- $absorbance_{sample}$ / $absorbance_{control}$] x100%.

### Total phenolics assay

Total phenolics were estimated as gallic acid equivalents essentially according to that described by Quettier-Deleu et al. (2000) with minor modification. An aliquot of 0.5 ml methanol extract was added to 7.0 ml deionized water and 0.5 ml Folin-Ciocalteu phenol reagent. After 3 min, 2.0 ml of 20% $Na_2CO_3$ were added and heated in a boiling water bath for 1 min comparatively to gallic acid standard. Absorbance was measured at 750 nm after cooling in darkness and the results expressed in mg of gallic acid/g dry okara.

### Statistical analysis

The mean value ± standard deviation was calculated from the data obtained from the three separate experiments. These data were analyzed statistically using one-way analysis of variance (ANOVA) followed by the student *t*-test.

## RESULTS AND DISCUSSION

### Nutritional quality of fermented okara

Previously, O'Toole (1999) reported that okara alone has some anti-nutritional 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 and it can reduce the level of free radicals in the body. In a related study, Zhu et al. (2008) reported that the okara with a low-protein-content byproduct of tofu, soymilk or soy protein was used as substrate to obtain a functional food by fermentation with *B. subtilis*.

The proximate composition of sterilized natural okara and fermented okara (different yeasts) were determined and nutritionally compared. The results in Table 1 revealed that, the constituents of sterilized okara on a dry matter basis (71.60% moisture) were 25.52% protein, 12% fat, 32.60% carbohydrate, 12.20% fiber and 3.96% ash. These results are nearly resembled to those values of fresh okara by Rashad et al. (2010).

The fermentation of okara with six different yeast strains improved the nutritional qualities or quantity of the okara, however, these varied from one organism to another (Table 1). The highest reduction of the crude fiber contents of the fermented okara were reduced by 45.50% in *C. albicans* and 38.36% in *K. marxianus* 8281, while the lowest reduction occurred with *K. marxianus* 7571 and *S. cerevisiae* by 7.38 and 7.70% respectively.

The reduction in the crude fiber contents of the fermented substrates is an indication of secretion of cellulose/hemicellulose-degrading enzymes by the yeasts during fermentation (Lateef et al., 2008). These data

**Table 1.** Approximate composition (g %) of the fermented and non-fermented sterilized okara.

| Sample | Crude fiber | Protein | Crude fat | Carbohydrate | Ash |
|---|---|---|---|---|---|
| Non-fermented okara | 12.20 | 25.52 | 12.00 | 32.60 | 3.96 |
| Fermented okara with: | | | | | |
| *Candida albicans* NRRL Y-12 | 6.65 | 37.07 | 10.97 | 19.78 | 4.95 |
| % Change | -45.50 | 45.26 | -8.60 | -39.33 | 24.99 |
| *Candida guilliermondii* NRRL Y-2075 | 10.24 | 36.74 | 10.37 | 22.54 | 4.40 |
| % Change | -16.07 | 44.00 | -13.60 | -30.86 | 11.10 |
| *Kluyveromyces marxianus* NRRL Y-7571 | 11.30 | 35.52 | 8.50 | 32.37 | 4.07 |
| % Change | -7.38 | 39.20 | -29.17 | -0.71 | 2.77 |
| *Kluyveromyces marxianus* NRRL Y-8281 | 7.52 | 30.64 | 11.50 | 23.74 | 4.51 |
| % Change | -38.36 | 20.10 | -4.17 | -27.18 | 13.88 |
| *Pichia pinus* | 11.07 | 39.41 | 9.68 | 23.14 | 4.18 |
| % Change | -9.26 | 54.43 | -19.33 | -29.02 | 5.55 |
| *Saccharomyces cerevisiae* NRRL Y-12632 | 11.26 | 38.45 | 11.60 | 15.95 | 5.06 |
| % Change | -7.70 | 50.67 | -6.67 | -51.07 | 27.77 |

% change represented as increasing or reduction (-) comparing to non-fermented (control).

suggest that individual yeasts may differ in their ability to modify soluble and insoluble fibers in the tested solid-state reaction systems. Moore et al. (2007) stated that, this reduction may be explained by the fact that individual yeast preparations may have different enzyme activities and interact differently with soluble and insoluble fiber components.

Several organisms including *A. oryzae*, *R. oligosporus* and baker's yeast have been reported to degrade cellulose/hemicellulose in similar manner (Matsuo, 1989a, b; Moore et al., 2007). Okara was fermented using the tempe fungus, *R. oligosporus* and the koji fungus *A. oryzae* (Matsuo, 1989a, b), to improve its nutritional qualities as a high-fiber, low energy foodstuff suitable for human food. Both fungi reduced the fiber content of okara (56.6 to 49.5%) than did the controls. In general, these results reported show that the two yeast strains *C. albicans* and *K. marxianus* 8281can effectively lower the crude fiber contents of the okara and this will improve their digestibility by animals.

The protein contents of the fermented substrates were promising increased in the range of (20.10 - 54.43%) as shown in Table 1. The results indicated that, solid-state yeast treatments significantly increased protein contents of okara which may refer to the rapid increase in growth of the yeasts using okara as a substrate as previously mentioned by Moore et al. (2007) on the fermented wheat bran by yeasts. Matsuo (1997) observed an increase in protein content of fermented okara from 22 to 27% using *Neurospora intermedia*. Also Iluyemi et al. (2006) reported real increase in the protein content of the fermented palm kernel cake in the range of (29.40-54.50%) by different fungal isolates.

Generally, fermentation of the okara led to reduction in the crude fat contents. The reductions were 8.60, 13.60, 29.17, 4.17, 19.33 and 6.67% in *C. albicans*; *C. guilliermondii*; *K. marxianus* 7571, *K. marxianus* 8281; *P. pinus* and *S. cerevisiae* respectively. In a similar study, the fat content of fermented okara by *N. intermedia* was reduced from 15 to 9% (Matsuo, 1997). Previous studies have shown reduction in the lipid content of different substrates fermented with different microorganisms (Das and Weeks, 1979; Iluyemi et al., 2006; Lateef et al., 2008). They attributed that to the accumulation of lipids by the fungal strains and during fungal processing, some lipolytic strains assimilate lipids from substrates for biomass production leading to a general reduction of the overall lipid content of the substrate.

The carbohydrate contents of treated and control okara samples were determined. Results in Table (1) indicated significant decreased in the value of carbohydrate contents of all the fermented okara samples except *K. marxianus* NRRL Y-7571. Matsuo (1989 a, b) reported that, the fermented okara by *R. oligosporus* and *A. oryzae* contained more free sugar (12 to 18%) than the controls.

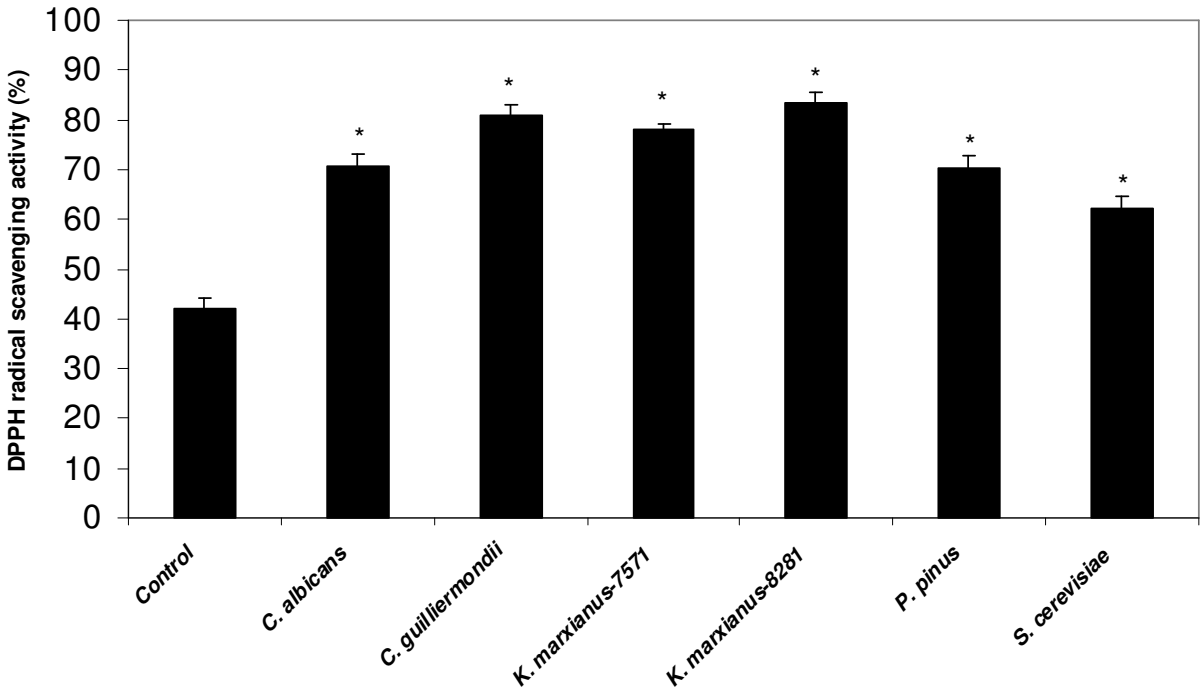


**Figure 1**. DPPH free radical scavenging effects of various extracts of fermented and non-fermented okara. Each value represents mean ± SD (n = 3). * Column indicates a significant difference from control.

The ash contents of all the fermented okara substrates were improved (Table 1). No significant difference was found between ash levels among okara samples treated with the six different yeasts. This indicates that all the yeasts used behaved similarly in increasing ash contents. Since the ash content determination is a measure of mineral levels in the substrates, it can be inferred that fermentation contributed to the higher levels of the minerals obtained. Similar improved levels of ash content, following fermentation have been reported by O'Toole (1999) using okara as a substrate.

From the above results, it is clear that the value of okara could be improved through yeast fermentation. The solid-state yeast treatment procedures developed in this study may have potential application in improving the bioavailable nutritional properties of okara.

## Antioxidant activities

### Scavenging of DPPH radical

Scavenging activity on DPPH radicals of various methanolic extracts of fermented okara were determined. It was found that all the extracts at a dosage of 2 mg/ml, showed various degrees of scavenging effect for DPPH radicals (62.31-83.30%) depending on the starter organism (Figure 1). In comparison, the control (non-fermented sterilized okara extract) showed a scavenging effect of 42% for DPPH-free radical.

These results are higher than that of 2 mg/ml α-tocopherol (48%) and comparable to that of ascorbic acid (87%). Obviously, there were more antioxidant components present in fermented okara than in non-fermented one, which could react rapidly with DPPH radicals, and reduce almost all DPPH radical molecules corresponding to available hydroxyl groups (Brand-Williams et al., 1995). This result reveals that fermented and non-fermented okara are free radical inhibitors or scavengers, acting possibly as primary antioxidants. They might react with free radicals, particularly of the peroxy radicals, which are the major propagatous of the autoxidation chain of fat, thereby terminating the chain reaction (Gordon, 1990; Shahidi et al., 1992). These results are similar with that of soybean kojis for various organisms (Lin et al., 2006) and fermented okara by *Bacillus subtilis* B2 (Zhu et al., 2008).

### Reducing activity

In the present study, assay of reducing activity was based on the reduction of $Fe^{3+}$/ferricyanide complex to the ferrous form in the presence of reductants (antioxidants) in the tested samples. The $Fe^{2+}$ was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986). The reducing power of the various fermented okara extracts and non-fermented

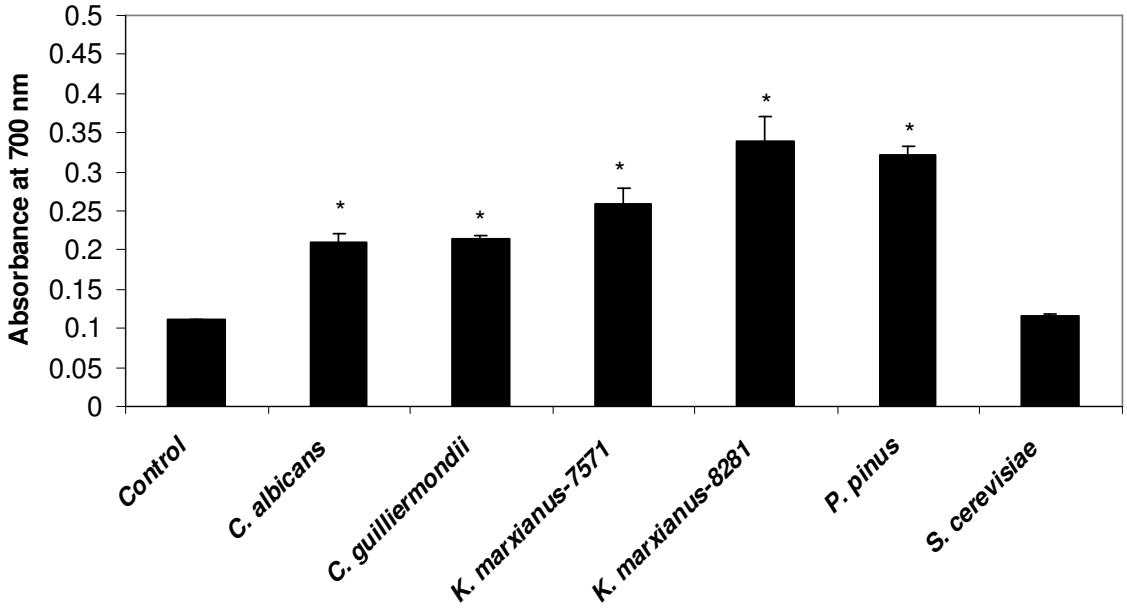


**Figure 2.** Reducing power of various extracts of fermented and non-fermented okara. Each value represents mean ± SD (n = 3). * Column indicates a significant difference from control.

one (control) is shown in Figure 2. All the fermented okara extracts showed a higher absorbance (0.115-0.340) than did the non-fermented okara extract (0.110) at the same dosage level (2 mg/ml). Extract of *K. marxianus* 8281 had relatively higher reducing power (0.340) than other extract (0.115-0.322).

Evidently, only fermented okara exhibited excellent reducing power. Fermented okara might produce certain metabolites with superior reducing power during fermentation, creating a major discrepancy between fermented okara and non-fermented okara. Similar findings of the enhanced reducing power of fermented bean and bean products have been previously reported (Berghofer et al., 1998; Yang et al., 2000; Chung et al., 2002; Wang et al., 2004; Zhu et al., 2008). The increased reducing power observed may be due to the formation of reductants that could react with free radicals to stabilize and terminate radical chain reactions during fermentation (Yang et al., 2000). In addition, the intracellular anti-oxidants, peptides of the starter organism and their hydrogen-donating ability may also contribute to this increased reducing ability (Yang et al., 2000).

### *β-Carotene/linoleic* acid system assay

All the tested samples exhibited good antioxidant activities ranged from 40 to 82% (Figure 3). This indicated that both fermented okara and non-fermented okara inhibited the peroxidation of linoleic acid at concentration of 2 mg/ml. The antioxidant activity of fermented okara and non-fermented okara might be due to the reducing of hydroperoxide, inactivating of free radicals, complexing with metal ions, or combinations thereof. This good antioxidant activity of okara might be attributed to the presence of phytochemicals, such as isoflavones (Wang and Wixon, 1999).

In general the linoleic acid radical scavenging activity of okara was significantly enhanced after fermentation by different yeasts comparing with the control. The anti-oxidant activity has been reported to be concomitant with the development of reducing power (Tanaka et al., 1988). However, this pattern was observed in this research.

Based on the results obtained, the marked inhibitory effect of fermented okara in the peroxidation of linoleic acid could in part be caused by its properties of scavenging free radicals and containing reductones. However, the inhibitory effect of non-fermented okara could in part be caused by its scavenging properties (Yang et al., 2000).

### Ferrous ion chelating ability

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Gordon, 1990). The catalysis of metal ions also correlates with incidents of cancer and arthritis (Halliwell et al., 1995). Ferrous ions, the most effective pro-oxidants, are commonly found in food systems (Yamaguchi et al., 1998). In the present study, the chelating ability of the okara extracts toward ferrous ions was investigated. As shown in Figure 4, all the methanol extracts tested exhibited $Fe^{2+}$-ion chelating effect. At the dosage level of

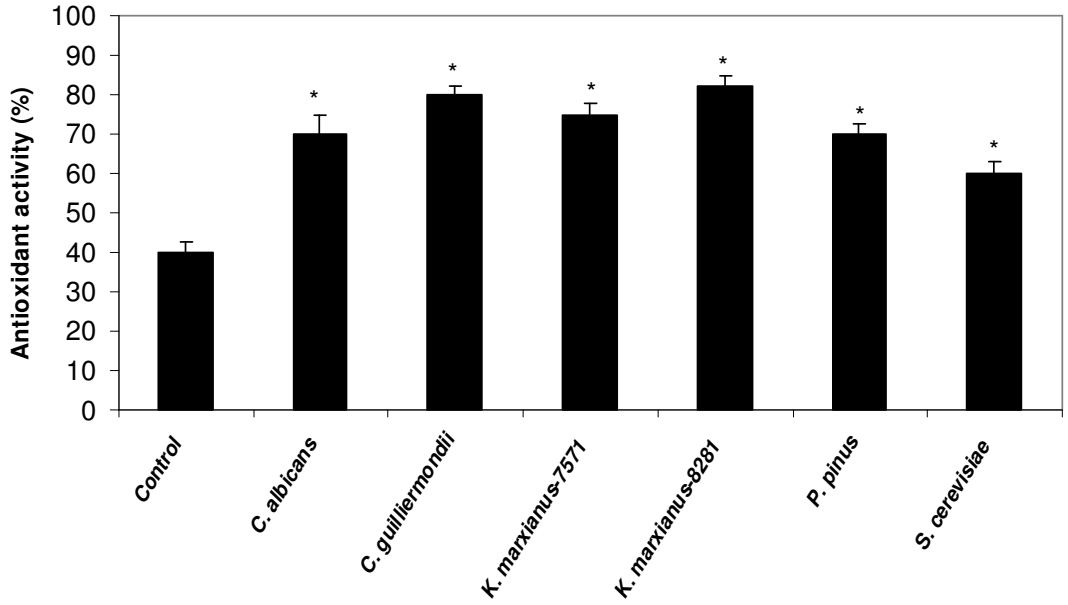


**Figure 3.** Antioxidant activity of various extracts of fermented and non-fermented okara in the β-carotene / linoleic acid system assay. Each value represents mean ± SD (n = 3). * Column indicates a significant difference from control.

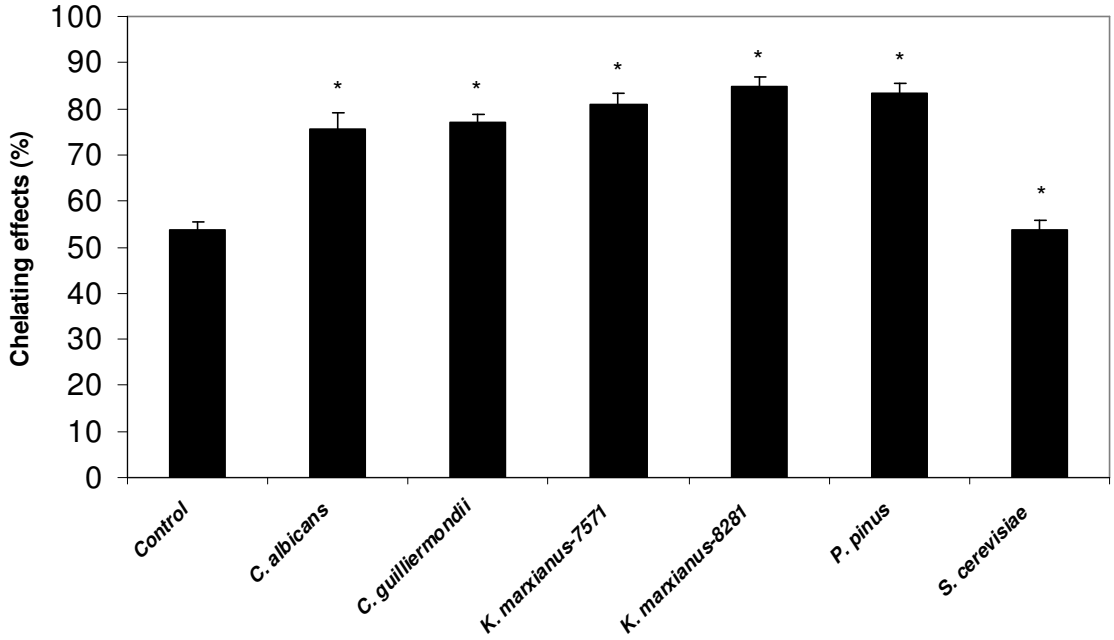


**Figure 4.** Chelating ability of various extracts of fermented and non-fermented okara to ferrous ion. Each value represents mean ± SD (n = 3). * Column indicates a significant difference from control.

2 mg/ml, the methanol extract regardless of their source, exhibited a chelating effect of 53.80-84.64% for $Fe^{2+}$ ions. In addition, extracts of all fermented okara, except that prepared with *S. cerevisiae*, showed a higher $Fe^{2+}$-ion-chelating effect than the extract of non-fermented one. The most $Fe^{2+}$-ion-chelating activity of *K. marxianus* 8281was methanolic extract (84.64%) and the lowest one was with *S. cerevisiae* (53.80%) which is the same level with the control. This indicated that both fermented and non-fermented okara were good chelators for ferrous ions. The value supported the result of Lin et al. (2006) where the methanol extract of all soybean koji regardless

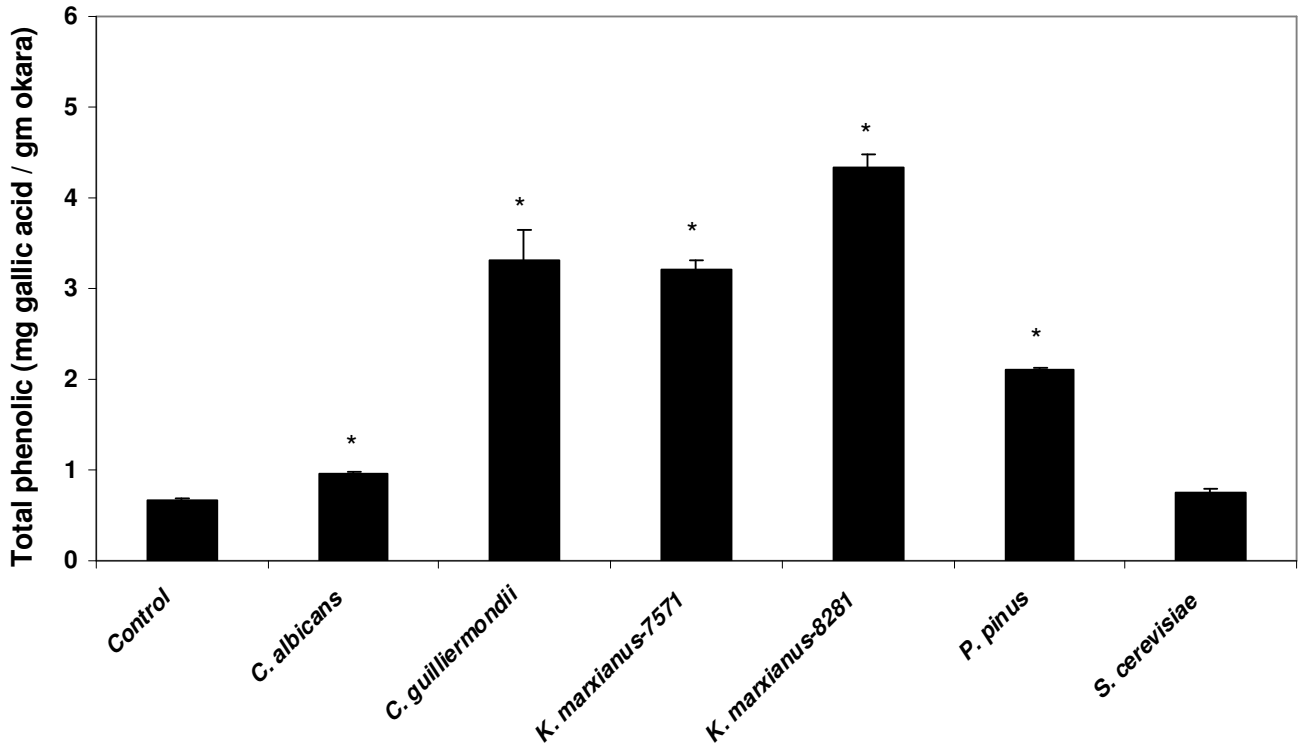


**Figure 5.** Total phenolic content of various extracts of fermented and non-fermented okara. Each value represents mean ± SD (n = 3). *, Column indicates a significant difference from control.

of their source exhibited a chelating effect of 84% or more for $Fe^{2+}$-ion at a concentration of 10 mg/ml. Besides that, the most extracts of soybean koji showed a higher chelating effect than the extract of non-fermented sterilized soybean.

## Total phenolic content

Phenolics are secondary metabolites, and in part, are produced as a result of the plant's interaction with the environment (Snyder and Nicholson, 1990). They have biological properties such as antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Han et al., 2007).

As shown in Figure 5, the total phenolic content of the methanol extract of the various fermented okara, depending on the starter organism, ranged between 0.74 and 4.50 mg gallic acid equivalents/g dry okara. All were higher than that of the control samples. In plants, phenolics are usually found in conjugated forms through hydroxyl groups with sugar as glycosides (Robbins, 1980). The increased in total phenolic content of fermented okara, observed in the present study, is consistent with the findings reported by other investigators (Vattem and Shetty, 2002; Randhir et al., 2004). These investigators suggested that, β-glucosidase produced by fungi, catalyse the release of aglycones from the bean substrate and thereby increase their phenolic content. Phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and metal-chelating ability (Shahidi et al., 1992; McCue and Shetty, 2003). Figure 5 shows that *K. marxianus* 8281 methanolic extract contains the highest amount of total phenolic compound among the various fermented okara extracts tested. The higher antioxidative activities observed with these fermented okara extracts (Figures 1 to 4) could thus relate to their high total phenolic content. Lin et al. (2006) reported that in comparison to the non-fermented soybean, the total phenolic content and antioxidative activities were increased maximum in soybean after fermentation with two different GRAS (general recognized as safe) filamentous fungi, *A. awamori* and *A. oryzae* BCRC 30222. Bhanja et al. (2009) demonstrated that fermented wheat grain is a better source of phenolics compared to non-fermented wheat.

## Conclusion

The present study has shown the potential use and

improves the health beneficial properties of soybean curd residue which are produced annually with large amount and of low utilization in Egypt by solid-state fermentation of six GRAS different yeast strains. The yeast strains improved the protein contents and the antioxidant activities of the soybean residue. They reduced the crude fiber contents of the substrates to varying degrees, while giving a good performance in its scavenging of free radicals. The yeast fermented waste may have potential application in improving its bio-available nutraceutical and nutritional properties. Further-more, the enrichment of this substrate, particularly in protein content and anti-oxidant activities may reduce the level of fortification in the preparation of animal feeds as it is done at present, thereby reducing the cost of producing the feeds.

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