Production of isoflavones-enriched soy yogurt through soymilk fermentation using probiotic bacteria

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Background and objective
Fermented soy products were regarded as healthy foods and hence are considered an essential part of the diet. Lactic acid bacteria isolated from naturally fermented Egyptian food products were screened for their ability to produce β-glucosidase, isoflavone aglycone, phenolics, and antioxidant activity during the formation of soy yogurt. The present research is a preliminary attempt to ascertain soy yogurt production by different strains of lactic acid bacteria and their efficacy for the production of the aforementioned products.
Materials and methods
A total of 16 probiotic lactic acid bacteria were used for the preparation of soy yogurt and tested for their probiotic properties. Soymilk was prepared and inoculated (1% v/v) with the probiotic strains previously activated in the MRS medium. After fermentation, cell viability, pH, titratable acidity, total phenolic compounds concentration, antioxidant activity, isoflavones aglycone (daidzein and genistein), and extracellular and cell membrane-bound β-glucosidase activity were determined.
Results and conclusion
A total of 16 probiotic lactic acid bacteria were used for the preparation of soy yogurt. The final pH of the fermented soymilk ranged from 4.92 to 6.6, and their titratable acidity (lactic acid %) ranged from 0.5 to 0.99%. Changes in β-glucosidase, isoflavone aglycone, total phenolics content, and antioxidant activity during the formation of soy yogurt were determined. All bacterial isolates showed positive cell-bound and extracellular β-glucosidase. Their activities ranged from 308.65 to 553 mU/ml. The *Lactobacillus* strains showed lower extracellular than their cell-bound β-glucosidase, and the opposite was true for the other group. An increase in the content of isoflavone aglycones in soy yogurt could be achieved by aging with bacterial fermentation. Soymilk fermented with *Lactobacillus* strains showed the highest bioconversion of isoflavone glucosides into isoflavone aglycones than other strains, although they produced less β-glucosidase enzymes. The antioxidant activity is related to changes in total phenolics. All microorganisms were able to increase the total phenols, whereas some *Lactobacillus* strains were unable to release more phenols compared with unfermented soymilk.

Keywords:
isoflavone aglycones, LAB, soy yogurt, β-glucosidase

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Introduction

The ingested nutrients have been considered as the dominant factor in the formation of the gut microbiota and tend to be more important in some cases than the host genetics [1]. The effect, on health, of gut microbiota has become a thrilling area of study [2]. Many studies have indicated that soy and soy product consumption may change the nature of gut microbiota and its population [3,4]. The intake of soymilk modified the bacterial composition, leading to a decrease in bifidobacteria and Firmicutes and a rise in the abundance of Bacteroidetes and Proteobacteria [4]. This change in the ratio of Firmicutes to Bacteroidetes was reported to affect metabolism with positive outcomes, resulting in a lower risk of obesity [1]. Soy and soy products are used as a good solution to address the drawbacks associated with dairy products. Preparation of probiotic-soy yogurt is a novel development in the field of fermented functional foods [5]. On May 22, 2020, GLOBE NEWSWIRE, New York reported that Reportlinker.com has announced the worldwide probiotics market is expected to expand by US$ 27.4 billion [6]. Probiotic-enriched food offers

a promising nonpharmacological approach to decrease the risk factors for hypercholesterolemia [7]. Probiotic bacteria improve gut health by inhibiting pathogenic gut bacteria's growth and attachment. Probiotics also work against some diseases such as hypertension, diabetes, cancer, allergy, and gout [8]. Donkor and Shah [9] stated that the growth of many probiotics is stimulated by fermented soymilk's protein. Fermented soy food offers certain extra benefits for the consumer because of its relation to the prevention of certain cancers, such as breast, prostate, and colon cancers. These effects are mainly owing to soy isoflavones. Soy isoflavones can modulate cell cycle, apoptosis, differentiation, proliferation, growth, and cell signaling [10]. Moreover, consumption of foods containing isoflavones can help maintain blood vessel health [11] and boost antioxidant levels that can promote cellular health [12]. Soy isoflavones are polyphenols with structure and chemical characteristics similar to those of 17-β-estradiol [13]. Isoflavones occur in unfermented soy products in the glycosidic form (daidzin, genistin, and glycitin), which have low bioavailability and estrogenic activity [14]. To achieve higher bioavailability and activity, aglycones (daidzein, genistein, and glycitein) must be released from their respective glycosides [15]. Such active form (aglycones) can be obtained by using β-glucosidases [16]. Fermentation of soy with microbial β-glucosidase results in products with enhanced isoflavone bioavailability [17,18]. Screening studies on the ability of lactic acid bacteria and bifidobacteria to hydrolyze isoflavone glycosides into their active form have been only undertaken for the past two decades [19]. In this manuscript, we are reporting on the ability of local isolated probiotic bacteria to ferment soymilk into soy yogurt. These bacteria have been tested for their β-glucosidase activity, the transformation of isoflavone glucosides into their active aglycones form, and their content. Growth of tested bacteria in soymilk, antioxidant activity, and titratable acidity of the produced soy yogurt were determined after fermentation.

Materials and method

Chemicals

Daidzein, genistein, *p*-nitrophenyl-β-D-glucoside (p-NPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteau reagent, and gallic acid were obtained from Sigma Company (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, and gallic acid. High-performance liquid chromatography grade methanol was purchased from Fisher Scientific (Hanover Park, Illinois, USA). Soybeans (glycine max) was purchased from the soybean unit, Agricultural Research Centre, Egypt. Nutrient agar medium was imported from Sisco Research Laboratories Pvt Ltd (SRL), New Mumbai, India, and DeMan, Rogosa, and Sharpe agar (MRS) broth were obtained from Laboratories Conda S.A., Madrid, Spain. All other chemicals used were of analytical grade. All chemicals used throughout the experiments were of analytical grades.

Microorganisms

A total of 16 probiotic strains [eight probiotic strains isolated from commercial dairy products (cheese and yogurt) and eight *Lactobacillus* spp. cultures previously isolated and identified by Negm El-Dein *et al.* [20]] were used throughout this study. *Lactobacillus casei* obtained from Chr. Hansen's Lab., Denmark, was used as a reference strain.

Isolation of probiotic bacteria

Isolation and culture conditions

Probiotic bacteria were isolated from dairy products, that is, cheese and yogurt, according to García-Hernández *et al.* [21] In brief, the dairy samples were homogenized in saline solution (NaCl, 0.9%), tenfold serially diluted, and plated on sterilized *Streptococcus thermophilus* agar medium. After an incubation period for 24–72 h at 37 and 45°C, colonies showing different morphologies were randomly selected. All isolates were subcultured and stored at −80°C in 50% (v/v) glycerol.

Preparation of soymilk

Soymilk was prepared according to Marazza *et al.* [22]. Whole soybeans were washed and soaked overnight in distilled water. The swollen soybeans were then ground with distilled water. The ratio of dry soybeans (125 g) to water (1 l) used for grinding was 1 : 8 (w/v). The slurry was filtered through double-layered cheesecloth to separate insoluble residues. The liquid was transferred into glass bottles and sterilized by autoclaving at 121°C for 15 min. The resulting soymilk was cooled and stored in a refrigerator (4°C) till use.

Soymilk fermentation

Overall, 100 ml of soymilk was inoculated (1% v/v) with the probiotic strains previously activated in the MRS medium. The inoculated soymilk was fermented at 40°C for 24 h. After fermentation, cell viability, pH, titratable acidity, total phenolic compound concentration, antioxidant activity, isoflavones, and extracellular, and cell membrane-bound β-glucosidase activity were

determined. Noninoculated soymilk incubated in the same experimental conditions was used as a control.

Analytical assays

Cell viability

Cell viability was determined by the plate dilution method using MRS and *S. thermophilus* agar media according to De Man *et al.* [23].

Assessment of β-glucosidase activity

Assessment of extracellular β-glucosidase activity: the β-glucosidase activity was determined using *p*-nitrophenyl-β-D-glucopyranoside as a substrate according to the method reported by Otieno and Shah [24] with some modifications. The reaction mixture in a total volume of 1.5 ml was composed of 0.5 ml of 0.1% *p*-nitrophenyl-β-D-glucopyranoside in 0.05 M sodium phosphate buffer with pH 7, and 1 ml of the culture supernatant (as a source of β-glucosidase enzyme). After 30 min of incubation at 37°C, 0.5 ml of 1 M cold sodium carbonate was added to stop the reaction. The aliquots were then placed in 2-ml Eppendorf centrifuge tubes and subjected to centrifugation at 15 000 rpm for 30 min using an Eppendorf centrifuge (model 5415D; Eppendorf, Hamburg, Germany). The amount of released *p*-nitrophenol was measured using a spectrophotometer (SP-2000UV; Spectra, USA) at a wavelength of 401 nm. A unit of enzyme activity was defined as the amount of enzyme that would liberate 1 mM *p*-nitrophenol per minute under assay conditions.

Assessment of cell-bound β-glucosidase activity

The assay of β-glucosidase activity was monitored according to Kuo *et al.* [25]. Overall, 1 ml of culture broth was centrifuged at 15 000 rpm for 5 min, then the supernatant was discarded and the cell pellets were washed with 0.05 M sodium phosphate buffer (pH 7.0). The cell pellets were resuspended in 0.5 ml of 0.5 M sodium phosphate buffer (pH 7.0) containing 0.1% *p*-nitrophenyl-β-D-glucoside and incubated at 37°C for 30 min. The reaction was stopped by adding 0.5 ml of 1 M sodium carbonate and centrifuged at 15 000 rpm for 10 min. This reaction supernatant was measured using a spectrophotometer at a wavelength of 401 nm. The amount of released *p*-nitrophenol was measured as described before.

Acid production

The pH of the soymilk and soy yogurt samples was measured by using a pH meter. Titratable acidity was determined by the methods of AOAC [26] using 0.1 N NaOH solution, and the result was expressed as % lactic acid.

Extraction of isoflavones

The extraction of isoflavones, including daidzein and genistein from fermented and unfermented soy yogurt, was performed using a modified version of the method described by Fukutake *et al.* [27]. One gram of the freeze-dried sample was added to 10 ml of 80% (v/v) aqueous methanol and extracted under agitation for 24 h at 28°C. The homogenates were centrifuged at 15 000 rpm for 30 min and the obtained filtrates (methanolic extracts) were used to determine daidzein and genistein concentrations and their antioxidant activity and phenolic content.

Assessment of the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging assay

The ability of the extracts to scavenge the DPPH radical was assessed spectrophotometrically according to Pyo *et al.* [28]. Overall, 20 µl aliquot of each extract was mixed in a test tube with 1.0 ml methanol containing 0.1 mM DPPH, which is a stable free radical and has a typical absorbance at 517 nm. The decrease in absorbance at 517 nm was measured at 100 min. The decreased absorbance of DPPH was calculated by the following equation:

$$\text{Scavenging activity (\%)} = 100-[(Abs_{sample}-Abs_{blank})\times 100/Abs_{control}].$$

Where $Abs_{control}$ is the absorbance of the DPPH solution without the addition of the sample, Abs_{sample} is the absorbance of the mixture solution containing both the sample and DPPH, and Abs_{blank} is the absorbance of the blank solution without DPPH.

Determination of total polyphenolic compounds

The total soluble phenolic content was determined according to the modified Folin–Ciocalteu method [29]. The reaction mixture was composed of 0.75 µl of the sample, 650 µl of distilled water, 200 µl of the Folin–Ciocalteu reagent, and 100 µl of 7.5% sodium carbonate solution. The solution was vortexed and allowed to stand for 2 h, then the blue color developed was measured spectrophotometrically at 765 nm. A standard curve was prepared with gallic acid of known concentrations to determine the total phenolic content as mg of gallic acid equivalents in doublets.

High-performance liquid chromatography analysis of isoflavones

High-performance liquid chromatography instrument Young Lin (Young Lin Cooperation, Seoul, South Korea) consists of a Reprosil-Pur Basic C18 5 µm (dimension: 250×4.6 mm) column and a UV detector

(λ_{max}=210 nm). Isocratic elution was used to isolate the isoflavones for detection. The mobile phase consisted of 100% methanol and 10 mM/l of ammonium acetate (60 : 40) containing 1 ml of trifluoroacetic acid per liter of solvent mixture. This was set at a flow rate of 1 ml/min according to the method by Kuo *et al*. [25] with some modifications. Injection volumes of isoflavone standards (daidzein and genistein) and the samples were set at 100 µl throughout the run time of 15 min. Single standards were prepared for peak identification. Isoflavone concentrations were calculated back to a dry basis (µg/g).

Statistical analysis

All values are means of two separate experiments. Statistical analysis was done using Minitab (V.17.3.1.). Comparisons were evaluated using one-way analysis of variance followed by Tukey's post-hoc comparisons. Differences were considered as statistically significant in the case of *P* value less than 0.05.

Results and discussion

Change in the pH and viable cell numbers

Acidification-related parameters during fermentation of soymilk by isolated bacteria, pH, and concentrations of lactic, as well as probiotic bacteria viability at the end of the fermentation process, were determined (Table 1). Fermentation at 37°C at different levels of initial viable counts and final viable counts resulted in nonsignificantly greater pH. The final pH ranged from 4.92 to 6.6. Farnworth *et al*. [30] reported that the final pH of the probiotic-fermented soy yogurt was higher than that of probiotic-fermented cow's milk yogurt, so the observed higher acidification in soy products indicates soy protein's lower buffering capability compared with milk proteins, affected mainly by proteins, acid/base groups, and organic acids. Proteins function as effective buffers because they contain both acid and base groups [31].

Titratable acidity (% lactic acid) of the fermented soymilk with different isolates ranged from 0.5 to 0.99%, whereas the highest value (0.99) was recorded by *Lactobacillus plantarum* KU985432 at 24 h of fermentation. In contrast, isolate E formed less acid in soymilk.

A maximum increase of percentage in final cell counts was recorded with bacterial isolate *L. plantarum* KU985432 and the lowest with isolated *Lactobacillus rhamnosus* KU985438. It seems therefore that the growth of bacteria causes the acidity of soy yogurt. The development of acid in the medium is affected

Table 1 Characteristics of soy yogurt prepared by fermented soymilk with isolated probiotic bacteria

Bacterial isolate	Final viable counts (CFU/ml)	Initial viable counts (CFU/ml)	Final pH	Titratable acidity (% lactic acid)
Lactobacillus plantarum KU985432	$(8\pm0^{ab})\times10^6$	0.512×10^6	4.92±0.01	0.99 ± 0.18^{a}
Lactobacillus plantarum KU985433	$(2.26\pm0.4^{efg})\times10^6$	0.318×10^6	5.31±0.01	0.83 ± 0.05^{abc}
Lactobacillus rhamnosus KU985434	$(0.9\pm0.1^{g})\times10^6$	0.847×10^6	5.35±0.31	0.67 ± 0.08^{abc}
Lactobacillus rhamnosus KU985435	$(7\pm1.4^{bcd})\times10^6$	0.289×10^6	5.26±0.02	0.76 ± 0.05^{abc}
Lactobacillus rhamnosus KU985436	$(2.32\pm0.1^{efg})\times10^6$	1.45×10^6	5.36±0.18	0.59 ± 0.03^{bc}
Lactobacillus rhamnosus KU985437	$(1.1\pm0.7^{fg})\times10^6$	1.237×10^6	5.53±0.46	0.63 ± 0.18^{bc}
Lactobacillus plantarum KU985438	$(1.04\pm0.2^{g})\times10^6$	0.914×10^6	5.28±0.02	0.68 ± 0.15^{abc}
Lactobacillus rhamnosus KU985439	$(4.2\pm0.3^{def*})\times10^6$	0.350×10^6	$5.92^{*}\pm0.03$	$0.61\pm0.1^{bc*}$
Isolate A (*Streptococcus* sp.)	$(6.3\pm0.64^{bcd})\times10^6$	2.5×10^6	5.92±0.02	0.79 ± 0^{abc}
Isolate B (*Bacillus* sp.)	$(7.5\pm0.71^{bc})\times10^6$	2.8×10^6	5.96±0.13	0.87 ± 0^{ab}
Isolate C (*Bacillus* sp.)	$(4.4\pm0.81^{cde})\times10^6$	2.0×10^6	6.01±0.33	0.87 ± 0^{ab}
Isolate D (*Bacillus* sp.)	$(8.5\pm0.71^{ab})\times10^6$	3.4×10^6	5.84±0.04	0.87 ± 0.1^{ab}
Isolate E (*Bacillus* sp.)	$(7.83\pm0.46^{ab})\times10^6$	3.75×10^6	6.59±0.05	0.50 ± 0^{c}
Isolate F (*Bacillus* sp.)	$(2.1\pm0.0^{efg})\times10^6$	1.20×10^6	5.79±0.01	0.90 ± 0.05^{ab}
Isolate G (*Bacillus* sp.)	$(6.03\pm1.45^{bcd})\times10^6$	2.0×10^6	6.36±0.06	0.72 ± 0^{abc}
Isolate H (*Bacillus* sp.)	$(6.9\pm0.53^{bcd})\times10^6$	2.57×10^6	5.87±0.11	0.87 ± 0^{ab}
Lactobacillus casei	$(54\pm1.7^{a})\times10^6$	0.389×10^6	5.24±0.06	0.61 ± 0.1^{bc}

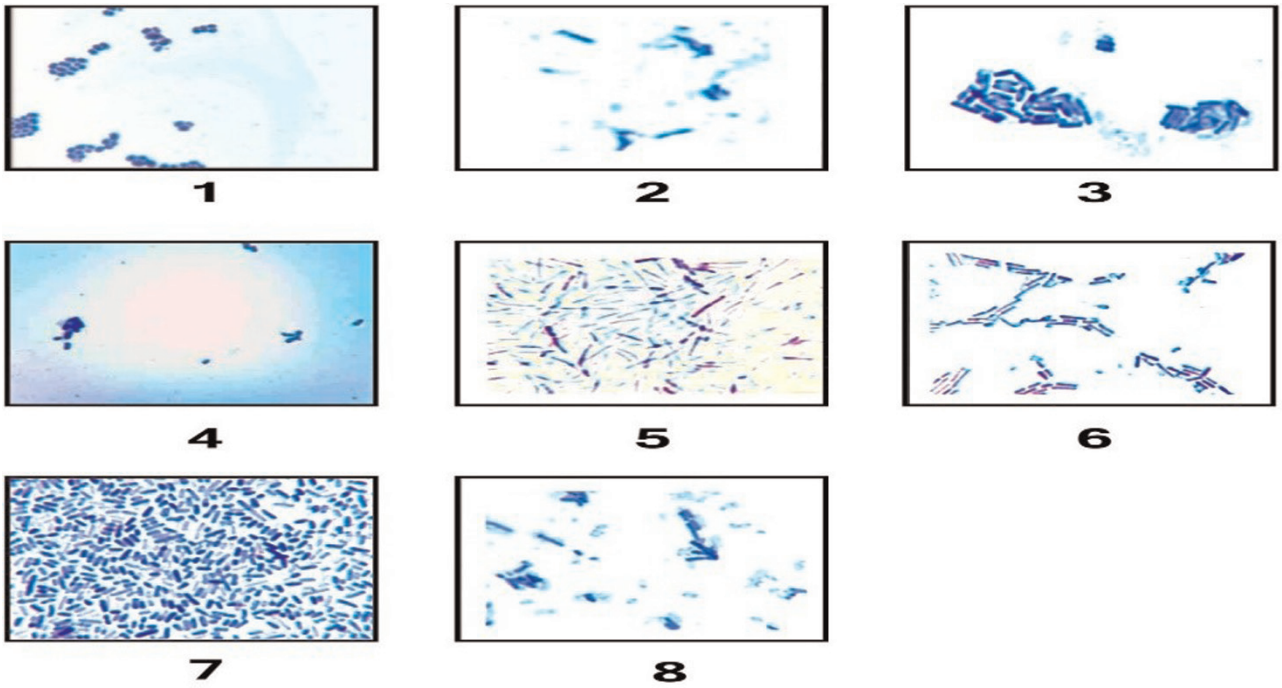
Values are mean±SD (*n*=2). Means that do not share a letter are significantly different.

largely mainly by the species of the organism and its capacity to ferment the medium's carbohydrates. Soy carbohydrates that can be fermented are characterized by water solubility and low-molecular-weight oligosaccharides such as sucrose (5.0%), raffinose (1.1%), and stachyose (3.8%) [32]. Angeles and Marth [33] stated that acid produced by different LAB in soymilk was not necessarily specifically associated with the growth rates of the tested bacteria. The authors mentioned that notable acid formation was restricted to the bacteria which could hydrolase soymilk's carbohydrates, for example, *S. thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus pentosus*, and *Leuconostoc mesenteroides*, and some LAB cannot utilize these carbohydrates. Moreover, *Lactobacillus fermentum*, *Streptococcus salivarius subsp. Thermophilus*, and *Bifidobacterium longum*, which hydrolyze soy carbohydrates, showed robust growth and formed high quantities of soymilk acids [34]. Bacterial growth in soymilk was different, with statistical significances observed for some strains (Fig. 1). It was higher than that of the initial one, indicating soymilk enhanced the growth of all tested probiotic cultures. In several nations, viable bacterial counts of yogurt should be between 10^6 and 10^9 CFU/ml. Soy yogurt developed in this study hence matches the standards for commercial yogurt. A near and reliable association between pH and titratable acidity is obvious. A slight change may be explained by variations in the types of strain and acid formed, for example, the proportion of weakly dissociated acids [33].

Changes in β-glucosidase and isoflavone aglycone contents during fermentation of soy yogurt

Microorganisms have different types of β-glucosidases: intracellular, extracellular, or cell-bound enzymes [35]. β-glucosidases produced by bacteria are parts of cellulosomes [36]. Most of the β-glucosidases produced by bacteria are intracellular and a member of GH 1 [37]. β-glucosidases of GH 3 are found as extracellular or cell bound, whereas those of GH 1 are primarily intracellular [38]. The β-glucosidase activities of the bacterial strains of fermentable soymilk are summarized in Table 2. All bacterial isolates showed positive cell-bound and extracellular enzymes. Results are depicted; great differences in enzyme activity were recorded among the tested bacteria. The β-glucosidase activity of fermented soymilk was significantly different ($P<0.005$). In general, higher total enzyme activities were recorded for some strains (A, B, C, D, and H). Their activities ranged from 308.65 to 553 mU/ml, in which two bacterial isolates, A and B, showed significantly the highest activity of β-glucosidase relative to all other bacteria. Bacterial isolates F and G showed the lowest enzyme activity. The extracellular β-glucosidase enzymes produced from the first group (*L. plantarum*

Figure 1



Gram staining of isolated probiotic bacteria: 1: isolate A, 2: isolate B, 3: isolate C, 4: isolate D, 5: isolate E, 6: isolate F, 7: isolate G, 8: isolate H.

Table 2 Concentration of extracellular and cell-bound β-glucosidases produced by probiotic bacteria in soy yogurt

Bacterial isolates	Extracellular β-glucosidase (mU/ml)	Cell-bound β-glucosidase (mU/ml)
Lactobacillus plantarum KU985432	43.33±1.83^{d}	79.54±9^{cde}
Lactobacillus plantarum KU985433	41.2±0.92^{d}	81.11±1.31^{cde}
Lactobacillus rhamnosus KU985434	66.94±10.61^{d}	107.87±17.2^{abcd}
Lactobacillus rhamnosus KU985435	43.33±3.93^{d}	82.13±7.7^{cde}
Lactobacillus rhamnosus KU985436	43.7±1.31^{d}	147.78±3.14^{ab}
Lactobacillus rhamnosus KU985437	44.54±2.75^{d}	79.26±3.7^{cde}
Lactobacillus plantarum KU985438	43.61±2.94^{d}	73.7±0.52^{def}
Lactobacillus rhamnosus KU985439	44.35±4.94^{d}	55.74±3.93^{efg}
Isolate A (*Streptococcus* sp.)	382.13±4.32^{a}	150.88±25.2^{a}
Isolate B (*Bacillus* sp.)	295.46±3.8^{b}	133.61±10.9^{ab}
Isolate C (*Bacillus* sp.)	224.26±0.52^{c}	84.39±12.11^{cde}
Isolate D (*Bacillus* sp.)	223.63±58.03^{c}	123.61±16.37^{abc}
Isolate E (*Bacillus* sp.)	363.61±26.06^{a}	24.72±1.05^{g}
Isolate F (*Bacillus* sp.)	49.26±0.79^{d}	44.63±9.69^{efg}
Isolate G (*Bacillus* sp.)	43.15±3.67^{d}	34.49±8.58^{fg}
Isolate H (*Bacillus* sp.)	243.61±0.39^{bc}	122.27±0.46^{abc}
Lactobacillus casei	42.41±2.6^{d*}	103.43±17.2^{bcd*}

Values are mean±SD (n=2). Means that do not share a letter are significantly different.

KU985432, *L. plantarum* KU985433, *L. rhamnosus* KU985434, *L. rhamnosus* KU985435, *L. rhamnosus* KU985436, *L. rhamnosus* KU985437, *L. plantarum* KU985438, *L. rhamnosus* KU985439, and the standard strain *L. casei*) are lower than their cell-bound β-glucosidase, whereas the other group (A, B, C, D, E, F, G, and H) produced more extracellular enzymes than their cell-bound β-glucosidase. In agreement with previous reports [39], the fermented soymilk showed different levels of β-glucosidase activity based on the initiating microorganism. β-glucosidases produced intracellularly by many microorganisms usually showed broad substrate specificity [40]. Kuo *et al.* [25] found that soy fermented with *Bacillus* strains increased aglycone content. They also noted that β-glucosidase is a crucial enzyme for the conversion of soy glycosides into aglycones by deglycosylation. Isoflavonoids are diphenolic secondary metabolites of plants [41].

Isoflavones are abundantly found in soybeans and with less amount in others [42]. Isoflavones have been associated with several health benefits. During fermentation, isoflavone glycosides are transformed to aglycons with a higher phytoestrogen activity [42]. The isoflavone aglycones content obtained after 24 h fermentation of soymilk is reported in Table 3. Small quantities of both genistein (44.85±14.43 μg/g) and daidzein (48.1±3.8 μg/g) were detected in the control unfermented soymilk. Different daidzein and genistein yields were observed, ranging between 42.75 ±3.47–507.45±86.94 and 18.99±0.32–213.3±3.9 μg/g, respectively. In general, the amount of daidzein and genistein increased by about 10.4 and 4.7-folds during soymilk fermentation into yogurt fermentation from an initial amount of 48.1 and 44.85 μg/g, respectively. The daidzein yield was the highest (P<0.05) in *L. rhamnosus* KU985434 and *L. rhamnosus* KU985436, ranging from 224.9 to 507.5 μg/g, compared with *L. casei* (224.53 μg/g). The daidzein yield ranged between 96.01 and 148 μg/g in *L. plantarum* KU985432, *L. plantarum* KU985433, *L. rhamnosus* KU985435, *L. rhamnosus* KU985437, *L. rhamnosus* KU985438, and *L. rhamnosus* KU985439. Lower conversion (P<0.05) was obtained in isolates D and E. On the contrary, the genistein yield was highest (P<0.05) in *L. casei*, *L. plantarum* KU985432, *L. rhamnosus* KU985434, *L. plantarum* KU985438, and isolate F, ranging from 116 to 177.7 μg/g. The genistein yield ranged between 61.4 and 98.85 μg/g for *L. plantarum* KU985433, *L. rhamnosus* KU985437, and isolates A, B, C, D, G, and H, whereas lower production (P<0.05) was obtained in *L. rhamnosus* KU985435, *L. rhamnosus* KU985436, and *L. plantarum* KU985438. Generally, the amount of daidzein in fermented soymilk was higher than that of genistein for all stains except isolates C, D, E, and F. Wei *et al.* [43] suggested that daidzein produced in soybean fermentation with *B*acillus *subtilis* BCRC14718 was higher than genistein at 48 h. Soymilk fermented with Lactobacillus strains (*L. plantarum* KU985432, *L. plantarum* KU985433, *L. rhamnosus* KU985434, *L. rhamnosus* KU985435, *L. rhamnosus* KU985436, *L. rhamnosus* KU985437, *L. plantarum* KU985438, *L. rhamnosus* KU985439, and *L. casei*) showed the highest bioconversion of glucoside, whereas soymilk fermented with other LAB (A, B, C, D, E, F, G, and H) exhibited lower bioconversion after 24 h of

Table 3 Isoflavones content of the produced soy yogurt by isolated probiotic bacteria

Bacterial isolates	Isoflavones	
	Daidzein (μg/g)	Genistein (μg/g)
Lactobacillus plantarum KU985432	148±25.03^{bc}	121.54±48.84^{bcd}
Lactobacillus plantarum KU985433	128.62±0.85^{cd}	98.85±2.4^{cd}
Lactobacillus rhamnosus KU985434	224.91±2.76^{b}	213.3±3.9^{a}
Lactobacillus rhamnosus KU985435	137.38±20.62^{bcd}	24.98±0.17^{f}
Lactobacillus rhamnosus KU985436	507.45±86.94^{a}	31.63±0.97^{ef}
Lactobacillus rhamnosus KU985437	96.01±16.08^{cd}	89.9±3.24^{cde}
Lactobacillus plantarum KU985438	98.07±9.79^{cd}	116.39±26.44^{bcd}
Lactobacillus rhamnosus KU985439	107.94±18.49^{cd}	18.99±0.32^{f}
Isolate A (*Streptococcus* sp.)	65.09±1.49^{cd}	64.25±2.11^{def}
Isolate B (*Bacillus* sp.)	68.25±1.9^{cd}	64.15±0.01^{def}
Isolate C (*Bacillus* sp.)	60.32±3.78^{cd}	61.65±2.23^{def}
Isolate D (*Bacillus* sp.)	57.61±8.3^{cd}	62.13±7.36^{def}
Isolate E (*Bacillus* sp.)	42.75±3.47^{d}	61.43±1.73^{def}
Isolate F (*Bacillus* sp.)	141.52±0.57^{bc}	144.55±2.01^{bc}
Isolate G (*Bacillus* sp.)	68.99±7.08^{cd}	65.87±3.79^{def}
Isolate H (*Bacillus* sp.)	68.48±6.64^{cd}	64.08±4.15
Unfermented soymilk	48.1±3.8	44.85±14.43
Lactobacillus casei	224.53±8.12^{b*}	177.73±33.19^{ab*}

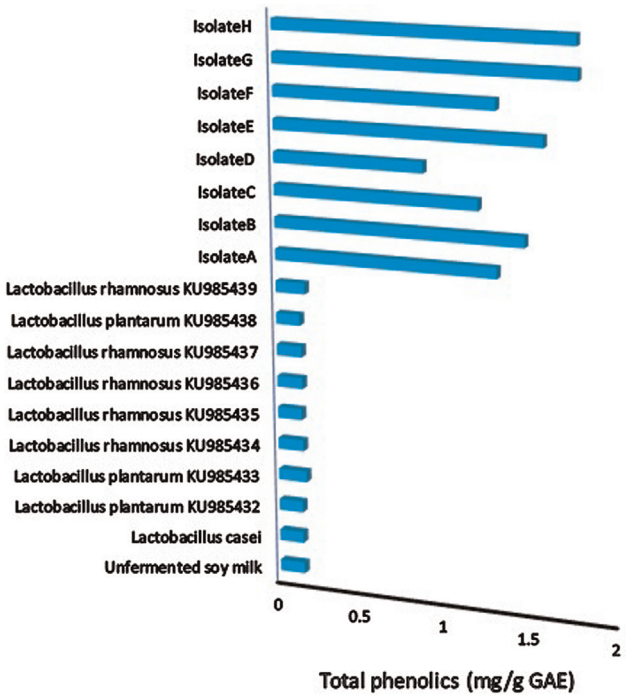
*Values are mean±SD (n=2). Means that do not a letter are significantly different.

fermentation, although they produced more β-glucosidase enzymes. Donkor and Shah [9] mentioned Lactobacillus *acidophilus* had a higher transformation rate of soymilk glycosides into aglycones than that of *Bifidobacterium lactis* and *L. casei* at 36 h of fermentation. In this work, soymilk has been used as a growth medium for LAB, whereas soybean paste has been used in previous studies (Doenjang), such as by Jung *et al.* [44], and soybean extracts in the study by Ketudat Cairns and Esen [36]. The conversion of glycosides to aglycones differed in soymilk fermented by probiotic cultures including bifidobacteria [28,45]; however, *B. longum* R0175 was unable to hydrolyze isoflavone glycoside at all [17].

Total phenolic content and antioxidant activity 2,2-diphenyl-1-picrylhydrazyl radical scavenging

Soymilk after fermentative bioprocesses was assessed for total phenolic content and DPPH radical scavenging activity. The data (Fig. 2) indicated all microorganisms could increase the total phenolic content. The maximum levels were observed for isolates A, D, C, D, E, F, G, and H. After fermentation, the contents of total phenolics were 577 to 1146.6% higher in fermented soy yogurts than those originated in their soymilk, respectively. However, the other *Lactobacillus* strains were unable to release more phenols compared with unfermented soymilk, except for *L. plantarum* KU985433 and *L. rhamnosus* KU985439. Such increase of total phenolics in soymilk after fermentation in this work is similar to the observations of other studies [46]. Microorganisms during fermentation produced enzymes that

Figure 2



Amount of total phenolic compounds in soy yogurt produced by different bacterial isolates.

hydrolyzed complex phenolics (β-glycosidase) and proteins (proteases) into their simpler forms, increasing concentration of total phenolics, amino acids, and peptides [47]. A similar observation was reported by Subrota *et al.* [48] in fermented soymilk with different Lactobacilli. They also reported that β-glucosidase produced by that bacteria is crucial in

Table 4 Antioxidant activity of the produced soy yogurt

Bacterial isolates	Antioxidant activity (%)
Lactobacillus plantarum KU985432	41.77±2.23^{fg}
Lactobacillus plantarum KU985433	41.27±2.84^{fg}
Lactobacillus rhamnosus KU985434	42.78±1.8^{fg}
Lactobacillus rhamnosus KU985435	47.34±2.13^{ef}
Lactobacillus rhamnosus KU985436	49.73±0.82^{de}
Lactobacillus rhamnosus KU985437	51.32±0.33^{cde}
Lactobacillus plantarum KU985438	39.49±0.76^{qw}
Lactobacillus rhamnosus KU985439	49.46±2.4^{de}
Isolate A (*Streptococcus* sp.)	46.91±0.33^{ef}
Isolate B (*Bacillus* sp.)	55.84±0.28^{bcd}
Isolate C (*Bacillus* sp.)	46.72±0.6^{ef}
Isolate D (*Bacillus* sp.)	56.53±0.06^{bc}
Isolate E (*Bacillus* sp.)	59.47±0.16^{ab}
Isolate F (*Bacillus* sp.)	39.34±1.31^{g}
Isolate G (*Bacillus* sp.)	65.27±0.16^{a}
Isolate H (*Bacillus* sp.)	40.27±0.22^{g}
Unfermented soymilk	32.23±1.63
Lactobacillus casei	45.56±3.56^{efg*}

*Values are mean±SD (n=2). Means that do not a letter are significantly different.

increasing the product's phenolic content. The scavenging effects of fermented soymilk on DPPH radicals were in the range from 39.49 to 65.27%, and strain G showed the highest scavenging capability, exhibiting approximately double the value of the control (32.23%) without fermentation. The data in Table 4 indicated the variations in antioxidant activity through fermentation were associated with variations in total phenolics. The antioxidant activity of soymilk has previously been reported to be increased by fermentation. Wang *et al.* [49] observed increased antioxidant activity of soymilk products fermented with LAB and bifidobacteria than unfermented soymilk. It has also been recorded that a positive correlation occurs between total phenolic content and increased radical scavenging activity after fermentation [50]. Marazza *et al.* [51] mentioned DPPH radical scavenging activity of soymilk increased after *L. rhamnosus* fermentation. Similar findings of increased radical scavenging activity after fermentation have been documented by other researchers [52]. In comparison, LAB also incorporates antioxidants on its cell surface and generates antioxidants, for example, peptides, L-3-(4-hydroxyphenyl) lactic acid, L-indole-3-lactic acid, and exopolysaccharides [53]. Although soymilk is a rich food containing not only many nutrients but also antioxidants such as isoflavones, saponins, vitamin E, peptides, and polyamines, higher functionality of fermented soymilk called soy yogurt maybe anticipated [54]. The antioxidant activity may outcome from the synergistic effects of other antioxidant components along with the phenolic content such as lipoproteins, free amino acids, and melanoidins, as already mentioned in fermented soy products [55]. Peptides and free amino acids in Chinese Douchi are also regarded as the origin of anti-free radicals and anti-linoleic acid oxidation [49].

Conclusion

This work was conducted to develop a local functional yogurt that delivers not only basic nutrition but also many health benefits. We produced soy yogurt by fermenting local soymilk by using local probiotic bacteria. The produced yogurt had many health characteristics as it can be used as an alternative to cow's milk yogurt. Such yogurt had active isoflavones (phytoestrogens), which have been considered as natural selective estrogen receptor modulators. Moreover, the yogurt exhibited higher antioxidant and phenolic compounds compared with unfermented soymilk. The significance of those results is that they demonstrate the potential benefits to people who experience allergy to cow's milk and those with estrogen-related diseases.

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Conflicts of interest

There are no conflicts of interest.

References

1 Carmody RN, Gerber GK, Luevano JM, Gatti DM, Somes L, Svenson KL, Turnbaugh PJ. Diet dominates host genotype in shaping the murine gut microbiota. Cell Host Microbe 2015; 17:72–84.

2 Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen gut microbiome axis: physiological and clinical implications. Maturitas 2017; 103:45–53.

3 Butteiger DN, Hibberd AA, McGraw NJ, Napawan N, Hall-Porter JM, Krul ES. Soy protein compared with milk protein in a western diet increases gut microbial diversity and reduces serum lipids in golden Syrian Hamsters. J Nutr 2016; 146:697–705.

4 Fernandez-Raudales D, Hoeflinger JL, Bringe NA, Cox SB, Dowd SE, Miller MJ, de Mejia EG. Consumption of different soymilk formulations differentially affects the gut microbiomes of overweight and obese men. Gut Microbes 2012; 3:490–500.

5 Mishra S, Mishra H. Technological aspects of probiotic functional food development. Nutrafoods 2012; 11:117–130.

6 Global Probiotics Industry. Probiotics market worldwide is projected to grow by US dollar 27.4 billion. [https://www.globenewswire.com/news-release/2020/05/22/2037833/0/en/Probiotics-market-worldwide-is-projected-to-grow-by-US-27-4-Billion.html]. 2020.

7 Islam SU. Clinical uses of probiotics. Medicine (Baltimore) 2016; 95:e2658.

8 El-Sayyad H. Future impact of probiotic therapy of metabolic disorder. J Pharma Rep 2017; 2:133.

9 Donkor ON, Shah NP. Production of beta-glucosidase and hydrolysis of isoflavone phytoestrogens by *Lactobacillus acidophilus, Bifidobacterium lactis*, and *Lactobacillus casei* in soymilk. J Food Sci 2008; 73:M15–M20.

10 Messina M, Kucuk O, Lampe JW. An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. J AOAC Int 2006; 89:1121–1134.

11 Messina M. Soybean isoflavone exposure does not have feminizing effects on men: a critical examination of the clinical evidence. Fertil Steril 2010; 93:2095–2104.

12 DiSilvestro RA, Mattern C, Wood N, Devor ST. Soy protein intake by active young adult men raises plasma antioxidant capacity without altering plasma testosterone. Nutr Res 2006; 26:92–95.

13 Andres S, Hansen U, Niemann B, Palavinskas R, Lampen A. Determination of the isoflavone composition and estrogenic activity of commercial dietary supplements based on soy or red clover. Food Funct 2015; 6: 2017–2025.

14 de Cremoux P, This P, Leclercq G, Jacquot Y. Controversies concerning the use of phytoestrogens in menopause management: bioavailability and metabolism. Maturitas 2010; 65: 334–339.

15 Islam MA, Bekele R, vanden Berg JHJ, Kuswanti Y, Thapa O, Soltani S, *et al.* Deconjugation of soy isoflavone glucuronides needed for estrogenic activity. Toxicol In Vitro 2015; 29:706–715.

16 Landete JM, Arques J, Medina M, Gaya P, de Las Rivas B, Munoz R. Bioactivation of phytoestrogens: intestinal bacteria and health. Crit Rev Food Sci 2016; 56:1826–1843.

17 Champagne CP, Tompkins TA, Buckley ND, Green-Johnson JM. Effect of fermentation by pure and mixed cultures of *Streptococcus thermophilus* and *Lactobacillus helveticus* on isoflavone and B-vitamin content of a fermented soy beverage. Food Microbiol 2010; 27:968–972.

18 Wei QK, Chen TR, Chen JT. Using of *Lactobacillus* and *Bifidobacterium* to product the isoflavone aglycones in fermented soymilk. Int J Food Microbiol 2007; 117:120–124.

19 Raimondi S, Roncaglia L, De Lucia M, Amaretti A, Leonardi A, Pagnoni UM, Rossi M. Bioconversion of soy isoflavones daidzin and daidzein by *Bifidobacterium* strains. Appl Microbiol Biotechnol 2009; 81:943–950.

20 Negm El-Dein A, Noor El-Deen A, Tolba S, El-Shatoury E, Awad G, Ibrahim M, Farid M. Probiotic properties and bile salt hydrolase activity of some isolated lactic acid bacteria. Egypt J Microbiol 2017; 52:87–100.

21 García-Hernández Y, Pérez-Sánchez T, Boucourt R, Balcázar JL, Nicoli JR, Moreira-Silva J, *et al.* Isolation, characterization and evaluation of probiotic lactic acid bacteria for potential use in animal production. Res Vet Sci 2016; 108:125–132.

22 Marazza JA, Garro MS, de Giori GS. Aglycone production by *Lactobacillus rhamnosus* CRL981 during soymilk fermentation. Food Microbiol 2009; 26:333–339.

23 De Man J, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli. J Appl Bacteriol 1960; 23:130–135.

24 Otieno DO, Shah NP. Endogenous beta-glucosidase and beta-galactosidase activities from selected probiotic micro-organisms and their role in isoflavone biotransformation in soymilk. J Appl Microbiol 2007; 103:910–917.

25 Kuo LC, Cheng WY, Wu RY, Huang CJ, Lee KT. Hydrolysis of black soybean isoflavone glycosides by *Bacillus subtilis* natto. Appl Microbiol Biotechnol 2006; 73:314–320.

26 AOAC: Official Methods of Analysis. Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement, vol. 15. Washington, DC: Association of Official Analytical Chemists; 1990.

27 Fukutake M, Takahashi M, Ishida K, Kawamura H, Sugimura T, Wakabayashi K. Quantification of genistein and genistin in soybeans and soybean products. Food Chem Toxicol 1996; 34:457–461.

28 Pyo YH, Lee TC, Lee YC. Effect of lactic acid fermentation on enrichment of antioxidant properties and bioactive Isoflavones in soybean. J Food Sci 2005; 70:S215–S220.

29 Parejo I, Viladomat F, Bastida J, Rosas-Romero A, Flerlage N, Burillo J, Codina C. Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. J Agr Food Chem 2002; 50:6882–6890.

30 Farnworth ER, Mainville I, Desjardins MP, Gardner N, Fliss I, Champagne C. Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. Int J Food Microbiol 2007; 116:174–181.

31 Mennah-Goveta YA, Singh RP, Bornhorst GM. Buffering capacity of protein-based model food systems in the context of gastric digestion. Food Funct 2019; 10:6074–6087.

32 Gerschenson LN, Rojas AM, Fissore EN. Chapter 3-carbohydrates. In: Galanakis CM, editor. Nutraceutical and functional food components. London, United Kingdom: Academic Press; 2017. PN 39–101.

33 Angeles AG, Marth E. Growth and activity of lactic-acid bacteria in soymilk. I. Growth and acid production. J Milk Food Technol 1971; 34:30–36.

34 Garro MS, Aguirre L, de Giori GS. Biological activity of *Bifidobacterium longum* in response to environmental pH. Appl Microbiol Biotechnol 2006; 70:612–617.

35 Günata Z, Vallier MJ. Production of a highly glucose-tolerant extracellular β-glucosidase by three Aspergillus strains. Biotechnol Lett 1999; 21:219–223.

36 Ketudat Cairns JR, Esen A. Beta-glucosidases. Cell Mol Life Sci 2010; 67:3389–3405.

37 Bhatia Y, Mishra S, Bisaria VS. Microbial beta-glucosidases: cloning, properties, and applications. Crit Rev Biotechnol 2002; 22:375–407.

38 Nijikken Y, Tsukada T, Igarashi K, Samejima M, Wakagi T, Shoun H, Fushinobu S. Crystal structure of intracellular family 1 beta-glucosidase BGL1A from the basidiomycete *Phanerochaete chrysosporium*. FEBS Lett 2007; 581:1514–1520.

39 Tsangalis D, Ashton J, McGill A, Shah N. Enzymic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase-producing bifidobacteria. J Food Sci 2002; 67:3104–3113.

40 Wołosowska S, Synowiecki J. Thermostable β-glucosidase with a broad substrate specifity suitable for processing of lactose-containing products. Food Chem 2004; 85:181–187.

41 Yang S, Wang L, Yan Q, Jiang Z, Li L. Hydrolysis of soybean isoflavone glycosides by a thermostable β-glucosidase from Paecilomyces thermophila. Food Chem 2009; 115:1247–1252.

42 Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet Gynecol 1996; 87: 897–904.

43 Wei QK, Chen TR, Chen JT. Use of *Bacillus subtilis* to enrich isoflavone aglycones in fermented natto. J Sci Food Agr 2008; 88:1007–1011.

44 Jung WY, Jung JY, Lee HJ, Jeon CO. Functional characterization of bacterial communities responsible for fermentation of Doenjang: a traditional Korean fermented soybean paste. Front Microbiol 2016; 7: 827.

45 Chen TR, Su RQ, Wei QK. Hydrolysis of isoflavone phytoestrogens in soymilk fermented by *Lactobacillus* and *Bifidobacterium* cocultures. J Food Biochem 2010; 34:1–12.

46 Moktan B, Saha J, Sarkar PK. Antioxidant activities of soybean as affected by *Bacillus*-fermentation to kinema. Food Res Int 2008; 41:586–593.

47 Sabokbar N, Khodaiyan F, Moosavi-Nasab M. Optimization of processing conditions to improve antioxidant activities of apple juice and whey based novel beverage fermented by kefir grains. J Food Sci Tech 2015; 52: 3422–3432.

48 Subrota H, Shilpa V, Brij S, Vandna K, Surajit M. Antioxidative activity and polyphenol content in fermented soy milk supplemented with WPC-70 by probiotic Lactobacilli. Int Food Res J 2013; 20: 2125.

49 Wang D, Wang LJ, Zhu FX, Zhu JY, Chen XD, Zou L, *et al.* In vitro and in vivo studies on the antioxidant activities of the aqueous extracts of Douchi (a traditional Chinese salt-fermented soybean food). Food Chem 2008; 107:1421–1428.

50 Vuong T, Martin L, Matar C. Antioxidant activity of fermented berry juices and their effects on nitric oxide and tumor necrosis factor-alpha production in macrophages 264.7 gamma NO(–) cell line. J Food Biochem 2006; 30: 249–268.

51 Marazza JA, Nazareno MA, de Giori GS, Garro MS. Enhancement of the antioxidant capacity of soymilk by fermentation with *Lactobacillus rhamnosus*. J Funct Foods 2012; 4:594–601.

52 Dordevic TM, Siler-Marinkovic SS, Dimitrijevic-Brankovic SI. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chem 2010; 119: 957–963.

53 Pessione E, Cirrincione S. Bioactive molecules released in food by lactic acid bacteria: encrypted peptides and biogenic amines. Front Microbiol 2016; 7:876.

54 Yamamoto N, Shoji M, Hoshigami H, Watanabe K, Watanabe K, Takatsuzu T, *et al.* Antioxidant capacity of soymilk yogurt and exopolysaccharides produced by lactic acid bacteria. Biosci Microbiota Food Health 2019; 38:97–104.

55 Delgado-Andrade C, Morales FJ. Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. J Agr Food Chem 2005; 53:1403–1407.