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Fermented Soybean Liquid Alleviated Peptic Ulcer through the Destruction of Acidic Proton Pump Rather than Suppression of Urease of *Helicobacter pylori*: A Kinetic Analysis

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ABSTRACT: Fermented soybean liquid (FSL) has been well cited for its broad spectrum of biological effects, yet its documented gastropeptic ulcer (GPU) ameliorating effect is still lacking. It was hypothesized that to avoid the injury exerted by gastric fluid, HP has to be sheltered in chyme emulsions immediately on infection. The HP urease (HPU) and the acidic proton pump (PP) may act as the "two-point pH modulator" to maintain an optimum pH between 6 and 7, and FSL is able to destroy such a modulating mechanism. FSL exhibited higher contents of isoflavonoids (2.5−17.3-fold) and essential amino acids (1.5−4.0-fold) than the nonfermented. FSL administered at 1 g/20 mL tid for 3 months eradicated *Helicobacter pylori* (HP) by 82% in 37 volunteers having GPU ($p < 0.20$); simultaneously, the plasma conjugated diene and TBARs levels were significantly resumed ($p < 0.05$). Kinetic analysis based on the conventional "urease theory" revealed that a cluster of 2.0×10^9 of HP cells is required for a single attack in the gastric lumen at pH 1.0−2.5. To verify the hypothesis, chyme-shelter testing was conducted in artificial gastric fluid (pH 2.4 ± 0.20). Results showed the HP cell viability was time- and size-dependent. At 20 min of contact time, the viability was 100, 4.2, 31.4, 43.3, 57.2, and 82.6%, respectively, in intact, dispersed, and particulate chymes (mesh sizes 80, 60, 40, and 20). The corresponding data became 96.2, 0.0, 14.5, 18.5, 21.3, and 28.6%, respectively, at a contact time of 40 min. Conclusively, the kinetic analysis and the chyme-shelter testing revealed that direct infection by bare HP cells is unlikely in real status. FSL is beneficial to GPU most probably due to its ability to raise blood alkalinity levels, destroying the PP and its ROS suppressing effect.

KEYWORDS: fermented soybean liquid, *H. pylori* urease, peptic ulcer, isoflavonoids, proton pump

INTRODUCTION

Accumulating evidence has linked the presence of *Helicobacter pylori* with the development of gastritis and peptic ulcer (PU).[1] *H. pylori* resides primarily in the gastric mucosa without invading the gastric epithelium, causing persistent low-grade gastric inflammation.[2] Normally, colonization of *H. pylori* in the duodenum is restricted to areas of gastric metaplasia and metaplastic gastric epithelium in the duodenal bulb of most patients with PU.[2] Currently, we have recognized that more than 90% of duodenal ulcers and 80% of gastric ulcers result from infection.[2] The prevalence of PU can be caused by (i) weakened intrinsic mucosal defensive factors, (ii) low protein diet supplement, or (iii) increased gastric secretion. These aggressive factors can enhance the invasive capability of *H. pylori*, promoting free radical attack and enhancing secretion of gastric acid, pepsin, bile salt, polypeptides, and hypercalcemia, leading to antral distension and alkalination (private communication from gastroenterologist Prof. Pan, Taipei Medical University, Taiwan). Radical attacks synergized by some atherosclerotic risk factors such as smoking, alcohol consumption, high-fat diet, lack of exercise, or high brain stress could emerge to elicit in vivo peroxidation of low-density lipoprotein (LDL), leading to accumulation of oxidized LDL (ox-LDL) in plasma.[3] Ox-LDL in turn can trigger a serial formation of inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 and -4, and interferon-γ, resulting in aggravation of gastric ulcer.[4]

Current treatments for *H. pylori* are becoming less effective due to mounting antibiotic resistance. A novel strategy suggests a treatment course of 7−14 days by "triple therapy", which usually comprises a proton-pump inhibitor and two antimicrobial agents, apparently more effective than the conventional "dual therapy".[5]

Soybean, *Glycine max* Merill, belongs to the family Leguminosae. It has been cultivated and utilized as food, nutrition, and medicine in the daily lives of the Chinese people for more than 5000 years. Soybean proteins (SBP) are a bile acid secretagogue and a hypolipidemic, as well as a strong LDL receptor activator.[6] Dietary protein peptic hydrolysate stimulated cholecystokinin release. Cholecystokinin secretion induced gastroprotection

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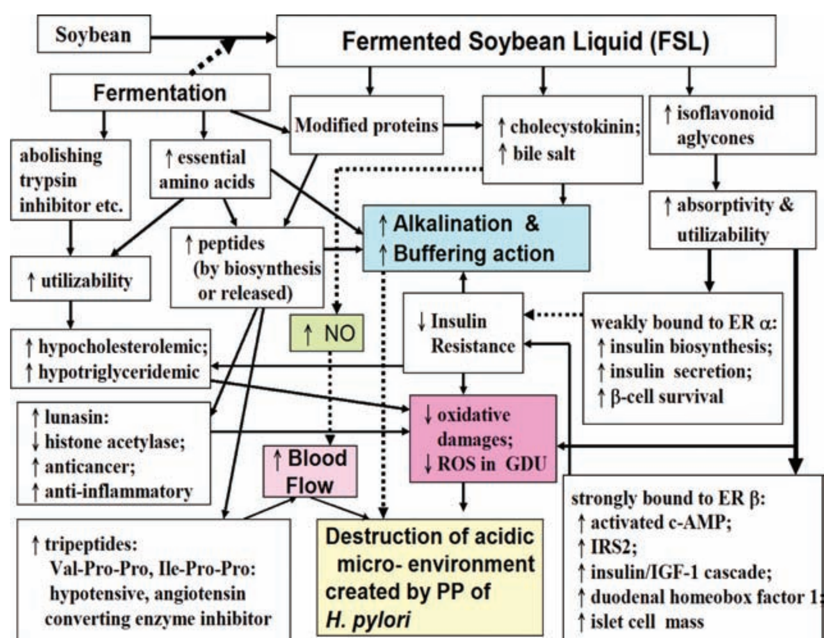

Figure 1. Hypothesized pharmacological potential of fermented soybean liquid for treatment of gastropeptic ulcer.

through the aid of nitric oxide and blood flow increase.[6] Moreover, fermented soybean liquid (FSL) contains significantly increased amounts of unique phytochemicals, isoflavonoids, especially genistein and daidzein,[7] which are more beneficial to health.

The therapeutic effect of FSL on PU is still lacking. We hypothesize that FSL may enhance intrinsic mucosal defensive factors by providing sufficient protein, bioactive peptides, and amino acid supplement through increased blood flow, alleviating ROS induced by insulin resistance and PU, suppressing hypercholesterolemic and hypertriglyceridemic risk factors, activating some antioxidative signaling pathways, and modifying the acidic microenvironment that otherwise would be favorable to the proliferation and invasiveness of *H. pylori* (Figure 1). To confirm this, we compared the constituents before and after fermentation and conducted this experiment with FSL on 37 patients exhibiting symptoms of PU.

MATERIALS AND METHODS

Chemicals. Genistein and genistin were isolated from a concentrate prepared from soy molasses by Protein Technologies International (St. Louis, MO) as directed by the manufacturer. Soybean lecithin and daidzein were purchased from LC Laboratories (Woburn, MA). Biochannin A, equol, dihydrodaidzein, *O*-demethylangolensin (ODMA), enterolactone, enterodiol, and other chemicals used were provided by Sigma-Aldrich Chemical Co. (St. Louis, MO).

Strains. *Aspergillus oryzae* (koji), *Saccharomyces rouzii*, and *Pedicoccus halophilus* were purchased from the Bioresource Collection and Research Center (BCRC) of the Food Industrial Research and Development Institute (Hsin-Chu City, Taiwan). The isolate of *H. pylori* (strain ATCC 43579, originated from human gastric samples) was obtained from the American Type Culture Collection (Manassas, VA).

Plate Cultivation of *H. pylori*. *H. pylori* was cultivated according to a method described by Kehler et al.[8] Briefly, Brucella broth with CO_2 plus sodium polyanetholsulfonate (SPS) (Becton Dickinson Microbiology Systems, Cockeysville, MD) was used for cultivation of *H. pylori*. Control organisms (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) were used for all blood culture systems. All isolates that had been stored at −70 °C were thawed for 3−4 days before inoculation. *H. pylori* was first plated on fresh *H. pylori* agars (89% Brucella agar, 10% defibrinated calf blood plus 1% IsoVitaleX) and incubated in a microaerobic atmosphere (CampyPak, BBL Microbiology system). Control strains were plated onto sheep blood and chocolate agar plates 1−2 days before inoculation (Remel, Lenexa, KS). Bacterial inocula were prepared by suspending a few colonies in phosphate-buffered saline (PBS) to a density of a 1.0 McFarland standard. Ten-fold dilutions were made, and 500 μL of a 10^{-5} dilution was inoculated into each flask. The size of each inoculation was determined by colony counts in triplicate, and the colony counts were averaged. Each blood culture bottle was also inoculated with 5 mL of fresh human blood from one of the healthy volunteers who had been determined to be *H. pylori* antibody negative. The blood culture bottles were ventilated according to the manufacturer's instruction to mimic clinical conditions and incubated under aerobic conditions at 37 °C, high relative humidity (RH ≥ 85%), and 5% CO_2. After inoculation, each bottle was routinely subcultured on days 1, 2, 3, 7, and 14. One hundred microliters of broth was plated onto commercially available chocolate and 5% sheep blood agars (Remel). Growth was recorded as positive if at least one colony of *H. pylori* was detected on any of the agars. *H. pylori* strains were further identified by morphology, and the identities were confirmed by positive urease, catalase, and oxidase tests. Control strains were identified by colony morphology, and the identities were confirmed by Gram staining, showing either Gram-positive cocci in clusters or Gram-negative rods. The actual growth in each blood culture bottle was performed for 72 h of incubation. The smears of culture were plated onto the *H. pylori* plates (Sigma-Aldrich) and incubated in CampyPak jars. The results were expressed as colony-forming units (CFU) per milliliter of broth. Geometric means for each time point were calculated by using Statgraphics 3.0 software (Graphics Software Systems, Inc., Rockville, MD).

Fermentation Brine. The fermentation brine consisted of (in g/L) NaCl, 2.5; KCl, 2.5; $Ni(NO_3)_2$, 0.01; $Co(NO_3)_2$, 0.01; $MgSO_4$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.3; $FeCl_2$, 0.1; Na_2HPO_4, 0.5; KH_2PO_4, $(NH_4)_2SO_4$, 1.5; $ZnSO_4$, 0.1; and $CuSO_4 \cdot 5H_2O$, 0.01. The brine was adjusted to pH 6.2 and autoclaved for 20 min at 121 °C.

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Preparation of Fermented Soybean Liquid. *G. max* Merill soybeans (SB) (4 kg), purchased from the local market of Taichung, were rinsed with distilled water twice, each time for 5 min to remove the adhering dust, and then the soybeans were steamed to remove microorganisms that adhere to the surface. The sterilized soybeans were smashed and ground into a pasty mass, and the fermentation brine was added at a ratio of 1.5 L/kg soybean and mixed well. The final volume was adjusted to 10 L with the sterilized brine. Koji, or *A. oryzae*, was inoculated at 5.0 g/L soybean. The process temperature was controlled within 33 ± 2 °C with the pH set at 6.0 ± 0.5. The starter fermentation was carried out for 5 days, and then *S. rouzii* 3.0 g/L and the bacterium *P. halophilus* 1.5 g/L were simultaneously inoculated into the fermentation liquid. The fermentation was continued for another 10 days at 30 °C and pH 5.1. The product was diluted with 20% fructose syrup to 50 g/L to make the FSL strength equivalent to 1.0 g/20 mL (FSL).

Analysis of Isoflavonoids by HPLC-MS. The isoflavonoids in sample ground SB paste and FSL were extracted with a 10-fold volume of 80% aqueous methanol (10 mL/g) as instructed by Barnes et al.[9] The extracts were repeated three times, and the combined extracts were filtered with a Whatman no. 2 filter paper. The filtrate was lyophilized and redissolved in deionized water while being kept warm in a 45 °C water bath. The solution was subjected to enzymic hydrolysis using mixed glucuronidase/sulfatase (Sigma-Aldrich). After incubation overnight at 37 °C, the aglycones were recovered by extraction with diethyl ether (IFVex). All analyses were analyzed by HPLC (Hewlett-Packard, Wilmington, DE; model 1050) linked to a PE-Sciex (Concorde, ON, Canada) API III triple-quadrupole mass spectrometer. Briefly, an Aquapore C_8 reversed-phase HPLC column (10 cm × 4.6 mm i.d., 300 Å pore size) was used for the separation of isoflavonoids and phytoestrogens in SB and FSL. The mobile elution was operated with a linear gradient of 0−50% acetonitrile in 10 mM ammonium acetate at a flow rate of 1 mL/min at pH 6.5 over 10 min. For mass determination, samples were introduced into the mass spectrometer via the HN-APCI interface operating in both the positive and negative modes. IFVex samples were separated by a reversed-phase HPLC column (l × i.d. = 15 cm × 0.21 cm). A Brownlee Aquapore C_8 column (Varian, Walnut Creek, CA) used a linear 0−50% gradient (5%/min) of acetonitrile in 10 mM ammonium acetate at pH 6.5 and a flow rate of 0.2 mL/min. The column elute was performed with a split of 1:1, one stream passed into the ion spray interface of the mass spectrometer operating in the negative ion mode, with an orifice potential of −60 V in the full scan mode; ions entering the mass spectrometer were analyzed over a m/z range from 50 to 800. MS/MS daughter ion spectra were obtained by passing the molecular ions selected by the first quadrupole into an argon gas collision cell, and the fragment ions were analyzed in a third quadrupole. Multiple reaction ion monitoring (MRM) was carried out in a similar manner to MS/MS by selection of specific ions not only in the first quadrupole but also in the third. Integration of peak area was carried out using the program MacQuan, provided by the mass spectrometer manufacturer (Hewlett-Packard). Areas were corrected by the peak area of the added internal standard biochannin A and compared to the areas of a set of known isoflavonoid standards to quantify the content of isoflavonoids in the IFVex samples.[9]

Amino Acid Analysis. The analysis of amino acids was performed as previously reported.[10] Briefly, 1 g of soybean was smashed and ground to a paste, to which 5 mL of phosphate buffer (pH 6.8) was added. The solution was heated at 80 °C for 10 min. The extract was filtered through a Whatman no. 2 filter paper. The extraction was repeated three times. The filtrates were combined and made up to 20 mL (SBE). Samples SBE and FSL, each 100 μL, were transferred into a 2 mL reaction vessel, to which 2 mL of 6 M HCl was added. The dissolved oxygen was purged off by nitrogen blowing for 10 min. The vessel was sealed, placed in the derivatization reactor, and heated at 110 °C for 24 h until the peptide moiety was completely hydrolyzed. The hydrolyzed product was lyophilized. The desiccated product was redissolved in 0.3 mL of 0.01 M HCl to obtain the sample amino acid mixture (AM).

Derivatization and Extraction. The authentic (0.3 mL) and AM (0.6 mL) solutions were respectively placed into 3 mL reaction vessels, to which the internal standard solution of norleucine (0.01 mL, 10 mg/mL) was added. After vigorous agitation, 0.1 mL of ethyl chloroformate and 1 mL of alcohol pyridine were added and mixed thoroughly. To the mixture was added 2 mL of chloroform. The solution was agitated for 1 min to accelerate derivatization and extraction, and 0.7 mL of water was added and shaken well. The reaction mixture was left to stand for 5 min to facilitate phase separation. The supernatant was discarded. The lower layer (i.e., the chloroform layer) was transferred into a new tube and dehydrated with 0.1 g of anhydrous sodium sulfate. The dehydrated chloroform extract was transferred into the sample vessel and analyzed by GC-MS.[10]

GC-MS Analysis. A GC-MS chromatograph (Agilent 6890, Wilmington, DE) installed with an FID detector and a column HP-5MS (l × i.d. = 30 m × 0.25 mm; film thickness, 0.25 μm) was used for GC-MS analysis. The operation conditions were as follows: flow rate of carrier nitrogen gas, 0.8 mL/min; operation temperature of detector FID, 305 °C; temperature of injection port, 300 °C. The elution temperature was programmed initially at 50 °C for 1 min and then raised at an elevation rate of 10 °C/min to 300 °C, at which it was held for 6.5 min.[10]

Detection of *H. pylori* by Noninvasive Stool Antigen Test. The experimental procedure was performed according to that of Koletzko et al. with slight modification.[11] Patients scheduled for endoscopy were asked to bring a stool sample of their own at the time of the procedure or to send it within 3 days after the endoscopy before any therapy was initiated. One hundred milligrams of feces (corresponding to two to five pellets, depending on the size) were collected and dissolved in 1 mL of 0.2% Tween 80 (Sigma-Aldrich, Bornem, Belgium) solution. The samples were vortexed vigorously until all fecal pellets were homogeneously suspended. After 10 min of sedimentation, the supernatant was first transferred to a 1.5 mL tube and centrifuged at 3800*g* for 1 min, and then the supernatant was transferred to a new 1.5 mL tube and centrifuged at 20800*g* for 5 min. The diluted stool supernatants (DSS) were collected and stored at −20 °C until analyzed. The stool antigen test was performed according to the manufacturer's recommendations using two different production lots. Those performing and reading the test were unaware of the *H. pylori* status of the patients tested. The stool antigen test is an enzyme immunoassay (EIA) that uses monoclonal mouse anti-*H. pylori* antibodies (Sigma-Aldrich, USA) adsorbed to microwells as capture antibody. First, 50 μL of supernatant of the DSS and thereafter 50 μL of conjugated monoclonal antibody solution were added to the wells and incubated for 1 h at room temperature on a shaker. Unbound material was removed by washing four times with a washing buffer. After washing, 100 μL of a substrate solution was added and incubated for 10 min. Then 100 μL of the termination solution was added, and the optical density was read by spectrophotometer at 450/630 nm dual wavelength. According to the manufacturer's guidelines, an optical density (OD) of <0.150 is defined as a negative and an OD of ≥0.150 as a positive test result. The test yielded a sensitivity of 98% and a specificity of 99%.[11]

Subjects. This experimentation was approved by the Human Medical Experiment and Ethics Committee of Human Experiment in Hungkuang University according to the Declaration of Helsinki (1979), and informed consent was obtained from each patient, if appropriate. In the beginning, 57 patients were collected. The exclusive conditions were (i) alcoholics, (ii) smokers, (iii) people who are taking NSAIDs, (iv) night workers, (v) cardiac diseased, (vi) chronic kidney diseased, (vii) psychological disturbances, and (viii) systolic blood pressure >160 mmHg and diastolic >110 mmHg. The inclusive requirement was (1) a positive breath test of *H. pylori.* In all 37 patients, the stool test detailed by Koletzko et al.[11] was performed instead of the conventional test

method for *H. pylori* status, which is carried out by biopsy-based methods (rapid urease test, culture, and histology) and/or UBT. Patients were excluded (i) if they had taken antibiotic or acid-suppressive drugs (proton-pump inhibitors, H2 receptor antagonists, antacids, bismuth preparations) within 4 weeks prior to testing, if they had received previous anti-*H. pylori* therapy, or if the *H. pylori* status was not clearly defined;[11] (ii) normal office hour workers; (iii) moderately stressed by their work; (iv) daily lunch hour not definite due to too heavy work loading; (v) a stage before blood stasis, that is, chronic ulcer (stage Ul-II—IV); or (vi) aged 30—55 years. Thus, the final participants were only 37 subjects. The placebo group comprising 16 members was given vitamin B complex one tablet uid only. The FSL-treated group having 21 members was given FSL orally at 20 mL tid.

Sera. The blood samples of the two groups were collected by venipuncture bleeding at months 0, 1, 2, and 2.5. The sample blood obtained was immediately centrifuged at 3000 rpm for 10 min, and the supernatant was separated and stored at −20 °C before use (plasma sample, PS). The LDL was separated as described in the following section.

Separation of LDL. The LDL samples were prepared according to modified method of Yamanaka et al.[3] The LDL fraction exhibiting a shining golden yellow color was obtained. For use in this study, the LDL was further dialyzed as directed. The final LDL obtained was determined for its protein content. Bovine serum albumin (BSA) was used as the reference standard to establish the calibration curve. The Bio-Rad protein agent was used for protein determination. On dilution to 8-fold, the diluted reagent (1 mL) was added to 2 μL of LDL, and on standing for 5 min, the absorbance was taken at 595 nm. The content of LDL was calculated against the BSA calibration curve. The remaining LDL was nitrogen gas filled and wrapped with aluminum foil to avoid direct light irradiation and stored at −20 °C. This LDL sample remains fresh for 1 week.

Detection of the Oxidative Status in LDL. *Determination of Conjugated Dienes.* The dialyzed LDL was adjusted with PBS for its content to make a concentration of LDL 125 μg protein/mL. The absorbance was read at 232 nm to examine the conjugated diene (CD) formation. The reference molar absorptivity of CD is $\varepsilon_{234} = 2.95 \times 10^4$ M^{-1} cm^{-1}.[3]

Determination of TBARs. The dialyzed LDL was adjusted with PBS for its LDL content to LDL 125 μg protein/mL. The following procedures were performed according to those of Hsieh et al.[3] Finally, the supernatant was separated, and the optical density was read at 532 nm. MDA was used as the reference compound, which on reaction with TBA has a molar extinguishing coefficient at 532 nm $\varepsilon_{532} = 1.56 \times 10^5$ M^{-1} cm^{-1}.[3]

Chyme-Shelter Testing. *Preparation of Artificial Saliva (ASL).* To 40 mL of 0.05 M Mes-Tris buffer (pH 8.2) were added 50 mg of mucin and 50 μL of thermostable R-amylase (EC 3.2.1.1, A3306, Sigma-Aldrich, USA).

Preparation of Artificial Gastric Fluid (AGF). The artificial gastric fluid has been prepared on the basic gastric fluid and the pepsin. The basic gastric fluid has been prepared according to the method of Clavel et al.[12] with some modifications. Briefly, 4.8 g of NaCl (Wako Pure Chemicals, Osaka, Japan), 1.56 g of $NaHCO_3$ (Wako Pure Chemicals), 2.2 g of KCl (Wako Pure Chemicals), and 0.22 g of $CaCl_2$ (Wako Pure Chemicals) were dissolved in 1 L of distilled water. After 15 min of autoclaving at 121 °C, the pH of the basic gastric fluid was adjusted to 2.4 ± 0.2 using 1 M HCl, and 2 mg of pepsin (Sigma-Aldrich, USA) per 50 mL of the artificial gastric fluid was added and mixed well (AGF). AGF was stored at 4 °C while not in use.

Preparation of Artificial Chyme Emulsion (ACE). The artificial diet was prepared by thoroughly blending 11 g of corn oil, 0.5 g of soybean lecithin, 30 g of cornstarch, 14 g of casein powder, 5 g of soy protein, 2 g of pectin, 2 g of mineral mix, 0.5 g of vitamin mix, and 10 g of desiccated spinach powder with 25 g of distilled water with a bench Blend YAP-214 (Taichung Fine Machinery Co., Taichung, Taiwan) for 30 min and separately passed through stainless sieve mesh sizes of 80, 60, 40, and 20 onto sterilized stainless pans, respectively. These screened particles were kept as loosely apart as possible in an aseptic room maintained at 4 °C (ACE).

Liquid Culture of H. pylori. The liquid culture medium was prepared according to the method of Xia et al.[13] with a slight modification. Briefly, to 90 mL of brain—heart infusion broth were added 5 mL of horse serum, 0.25 mL of yeast extract, 50 mg of vancomycin, and 50 mg of amphotericin B. Double-distilled water was added to make up the volume to 100 mL. The pH was adjusted to 6.5—7.0 with 1 M HCl. *H. pylori* ATCC 43579 was seeded at a density of 1×10^5 cells/mL and incubated at 37 °C under 5% CO_2 atmosphere for 48 h.

Preparation of H. pylori-Contaminated Chymes. To ACE (80 g) was added 120 mL of ASL. Onto the semisolid chymes was seeded *H. pylori* at a density 2×10^5 bacteria cells/mL. The mimic chymes were aseptically pasted with a sterilized pestle for 5 min to mimic the chewing process in the mouth. The chyme mass was separately sieved with an aseptic stainless screen to make particles of sizes 80, 60, 40, and 20 (particulate chyme groups, PCG).

Mimic Gastric Fluid Attack. To each 30 mL of AGF was added 2 g of original unscreened contaminated chyme mass, and PCG of sizes 80, 60, 40, and 20 were respectively added to aseptic 50 mL reaction beakers. The original unscreened contaminated chyme was used as the control. All other groups except the unscreened chyme mass remained unstirred for 60 min, whereas the original unscreened contaminated chyme mass was intermittently stirred at 60 rpm for 1 min within every 5 min cycle (designating the dispersed chyme to mimic the homogeneous state of chymes in stomach). The total treatment time lasted for 60 min.

Enumeration of the Survival of H. pylori. Percent bacterial survival was analyzed by two experiments, one performed in a similar fashion as described under Detection of *H. pylori* by Noninvasive Stool Antigen Test, starting from "The chyme antigen test was performed according to the manufacturer's recommendations using two different production lots...." The other was performed by the plate cultivation described under Plate Cultivation of *H. pylori*. The averaged data were taken.

Statistical Analysis. Data obtained in the same group were analyzed by Student's *t* test with computer statistical software SPSS 10.0 (SPSS, Chicago, IL). Statistical analysis system ANOVA with Tukey test software was used to analyze the variances and multiple range and test their significances of difference between paired means. A confidence level of $p < 0.01$ was for the highly significant difference and $p < 0.05$ for the significant difference, whereas a level of $p < 0.20$ was used to evaluate the therapeutic effect before and after the treatment with FSL.

RESULTS AND DISCUSSION

Proximate Composition. Fermentation initiated the degradation and biotransformation of carbohydrates, ptoteins, and lipids. The composition of carbohydrates, proteins, and lipids decreased from 27.6 to 11.8 g%, from 32.2 to 12.4 g%, and from 25.4 to 11.5 g%, respectively (Table 1). During fermentation, the environment becomes more suitable for salt-tolerant organisms such as *P. halophilus*.

Isoflavonoid Content Increased Significantly during Fermentation. Microbial fermentation transformed many isoflavonoids, including genistin, daidzin, genstein, daidzein, glycitin, and glycitein. The overall strength increased from 2.5-to17.3-fold. On the contrary, the content of malonylgenistin decreased significantly from 156.9 to 18.4 mg% (Table 2). During fermentation, 6-*O*-malonylglucosides are converted to

Table 1. Proximate Compositional Change before and after Fermentation[a]

item	contents (g%, g/100 g, dry basis)	
	before fermentation	after fermentation
carbohydrate	27.6 ± 3.2 a	11.8 ± 2.3 b
protein	32.2 ± 4.4 a	12.4 ± 2.2 b
lipid	25.4 ± 3.5 a	11.5 ± 2.3 b
total isoflavonoids	154.8 ± 22.5 a (dry basis)	48.7 ± 5.4 (wet basis)
		556.7 ± 26.8 b (dry basis)

[a] Data expressed as the mean ± SD from triplicate samples. Different letters in the same row indicate significant difference from each other ($p < 0.01$).

Table 2. Change of Isoflavonoid and Amino Acid Contents in Soybean Products before and after Fermentation[a]

compound	content (mg%)		compound	content (mg%)	
	before	after		before	after
Isoflavonoids					
genistin	46.7 ± 2.4	168.4 ± 37.4	daidzein	2.3 ± 0.6	12.7 ± 2.1
daidzin	35.8 ± 3.2	105.6 ± 17.4	glycitin	3.3 ± 0.7	10.2 ± 3.1
genstein	3.4 ± 0.6	27.7 ± 3.2	glycitein	0.4 ± 0.1	6.9 ± 2.2
malonylgenistin	156.9 ± 7.4	18.4 ± 2.5			
Amino Acids					
aspartic acid	1.6 ± 0.4	2.9 ± 1.2	valine	0.8 ± 0.2	3.2 ± 0.7
glutamic acid	4.3 ± 1.3	7.9 ± 1.7	leucine	1.7 ± 0.5	2.8 ± 0.6
serine	1.2 ± 0.3	1.8 ± 0.8	isoleucine	1.0 ± 0.3	3.8 ± 1.1
arginine	1.7 ± 0.4	1.9 ± 0.7	histidine	0.8 ± 0.2	2.4 ± 0.5
alanine	0.9 ± 0.2	1.2 ± 0.4	lysine	1.5 ± 0.5	2.2 ± 0.7
threonine	1.2 ± 0.5	1.6 ± 0.3	phenylalanine	1.2 ± 0.3	1.5 ± 0.4
cysteine	0.5 ± 0.2	0.4 ± 0.2	tyrosine	1.0 ± 0.4	2.3 ± 0.6
methionine	0.3 ± 0.1	5.0 ± 1.4	proline	1.2 ± 0.5	3.5 ± 0.8

[a] Data are expressed as the mean ± SD from triplicate samples.

6-*O*-acetylglucosides or β-glucosides by heat treatment or the action of β-glucosidases.[14] A summary of cited nutritional and biochemical aspects of fermented soybean is shown in Figure 1. Briefly, Yang et al. indicated that soy isoflavone aglycones such as genistein and daidzein are better absorbed than their corresponding glucosides[15] (Figure 1). Soybean isoflavonoid and protein consumption can alleviate insulin resistance and promote glycemic control[16,17] (Figure 1). Many soybean isoflavonoids exhibit estrogen-like structures.[18] Genistein and daidzein bind weakly to receptor α but more strongly to receptor β and, hence, can exhibit organ-specific estrogenic and antiestrogenic effects[19] (Figure 1).

Biological Effects of Soybean Protein, Peptides, and Amino Acids. Lunasin, a 43-amino-acid peptide from soybean, has anticancer and anti-inflammatory activities[20,21] (Figure 1). The tripeptides Val-Pro-Pro and Ile-Pro-Pro act as antihypertensive agents in spontaneously hypertensive rats[22] (Figure 1). The apparently increased contents of isoleucine, valine, and proline in FSL, respectively, from 1.0 to 3.8 mg%, from 0.8 to 3.2 mg%, and from 1.2 to 3.5 mg%, implicated that the transformation of Val-Pro-Pro and Ile-Pro-Pro in FSL is very likely (Table 2)[23] (Figure 1). We hypothesize that some of the above-mentioned pharmacological effects can be pertinently pointing to the destruction of the acidic microenvironment already built by the action of the proton-pump (PP) device of *H. pylori* when they have successfully penetrated into the gastric mucosa (Figure 1). Indeed, soybean-derived peptides currently have become the hot spot of investigation for new drugs and functional food ingredients for gut health and modulating the intestinal absorption of nutrients.

FSL Secured LDL Peroxidation. *Suppression of CD Formation.* The formation of conjugated dienes was greatly reduced by FSL administration (Table 3). Only 3 μM remained in the FSL group at the end of the experiment (Table 3). In LDL peroxidation, ox-LDL would first appear in the very early stage and be easily protonated. The latter is then dehydrated to form dienes, among which conjugated dienes are the most stable.[3]

Suppression of Conjugated TBARs Formation. As seen, FSL effectively suppressed the formation of TBARs. The FSL treatment showed a very low level of TBARs (38 μM) at the end of the 3 months of treatment (Table 3). In contrast, that of placebo consistently remained within 128−132 μM. In the early stage of LDL peroxidation, conjugated dienes usually first appear after a short period of lag time, followed by a serial subsequent oxidative process and then decomposed to produce a variety of decomposition products such as aldehydes and ketones.[3]

Na-Tou fungi, *Saccharomyces* sp., and *Asgergillus* sp. could produce a diversity of active antioxidative isoflavonoids through fermentation; all are good antilipid peroxidatives able to inhibit the oxidative modification of LDL by macrophages.[24] Polyphenolics prevented ROS damage, such as gastric hemorrhage, to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo.[25] Statistically, only LDL-cholesterol can be significantly affected in the *H. pylori*-infected tissue with greater disordered lipid metabolism ($p > 0.05$),[26] implicating the potential therapeutic effect of FSL antioxidants on GPU.

FSL Suppressed Growth of *H. pylori*. The resident number of *H. pylori* was attenuated by FSL treatment. The *H. pylori* count in the placebo remained almost unaffected within 52−57 CFU/mL, whereas 82% of the patients were greatly improved by FSL treatment (Table 3). Thus, the viability of *H. pylori* in gastric mucosa can be a major determinant for the prevalence of GPU. Tremendous clinical trials indicated that *H. pylori* eradication therapy can help gastric ulcer healing. Much of the literature has indicated that the pH value of the environment is the main determinant affecting the survival of *H. pylori*. The viability of *H. pylori* is not affected within pH 5−7; however, its growth and proliferation do not occur at pH ≤3 and ≥9, and its flagella proteins are inactivated at pH ≤3.0. Below pH 6.0, *H. pylori* organisms will survive but not divide.[27] Previously, a low-pH-inducible gene, *cagA*, has been shown to be relevant for the survival and persistence of *H. pylori* in the gastric environment.[28] *H. pylori* can survive in an environment having pH within 3.0−9.0, but its growth and proliferation can occur only within pH 6−8; in fact, the optimum pH range for its growth in gastric mucosa is 6.0−7.0[28] (Figure 2). Accumulating evidence elsewhere indicated that the *H. pylori* urease uniquely retards the acidic gastric fluid, so that they can survive in gastric lumen. *H. pylori* produces high-level HPU, which constitutes almost 6% of the soluble proteins.[29] The HPU secreted by *H. pylori* neutralizes gastric acid by decomposing urea to produce carbon dioxide and ammonia. The ammonia in turn neutralizes the gastric fluid to create a slightly acidic (pH 5.0−7.0) extracellular macroenvironment

Table 3. Change of *H. pylori* Count and LDL Oxidative Damages: TBARs and CD Formation in LDL Treated with FSL[a]

parameter	duration of treatment			
	0 months	1 month	2 months	3 months
H. pylori (CFU/mL) (%)				
placebo	56 ± 9 a,A (100%)	57 ± 11 a,B (100%)	54 ± 8 a,B (100%)	52 ± 12 a,B (100%)
FSL	57 ± 10 d,A (100%)	40 ± 11 c,A (100%)	12 ± 3 b,A (23%)	8 ± 2 a,A (18%)
CDs[b] (μM)				
placebo	25 ± 9 a,A (100%)	26 ± 5 a,B (100%)	24 ± 8 a,B (100%)	26 ± 4 a,B (100%)
FSL	25 ± 9 d,A	12 ± 4 9 c,A	5 ± 2 b,A	3 ± 2 a,A (100%)
TBARs[b] (μM)				
placebo	128 ± 49 a,A (100%)	134 ± 36 a,B (100%)	130 ± 44 a,B (100%)	132 ± 36 a,B (100%)
FSL	132 ± 33 d,A	112 ± 25 c,A	58 ± 11 b,A	38 ± 8 a,A (84%)

[a] Samples of LDL (125 μg protein/mL) were obtained at months 0, 1, 2, and 3, respectively, from the placebo and the FSL-treated groups. Upper case letters designate significant difference between the placebo and the FSL-treated parameter in each column. Lower case letters show significant time-dependent differences among data in each row. [b] Reference molar absorptivity of TBARs at 532 nm $\varepsilon_{532} = 1.56 \times 10^5$ M^{-1} cm^{-1}.[3] Reference molar absorptivity of conjugated diene (CD) at 234 nm is $\varepsilon_{234} = 2.95 \times 10^4$ M^{-1} cm^{-1}).[3].

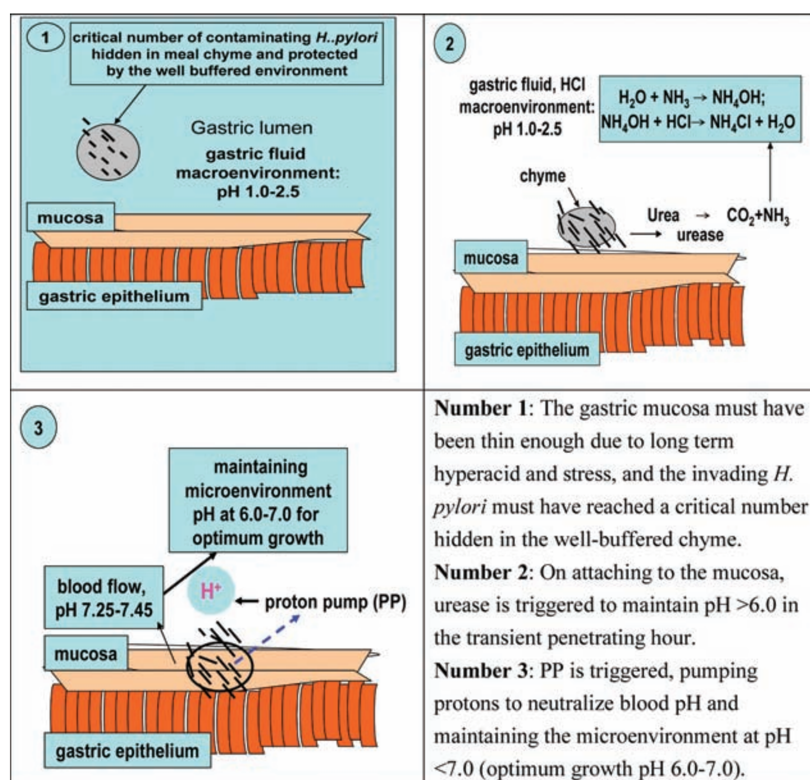


Figure 2. Role of urease and proton pump in colonization of *Helicobacter pylori* inside gastric mucosa.

(Figure 2) to accommodate a transient “favorable” environment for penetration. Unlike that of other urease-positive bacteria being only localized in plasma, HPU simultaneously appears in the cytoplasm and on the cell surface.[29,30] The HPU, a supramolecule comprising 12 copies of two subunits having molecular weights 61 and 27 kDa, respectively, coexists as $\alpha_{12}\beta_{12}$ with a low K_m value. Such a cluster of supramolecular assembly is crucial for the survival of the enzyme HPUre at low pH, >5.0; at pH <5.0, the enzyme is inactivated irreversibly.[31] The question now arises, how can *H. pylori* successfully withstand the high-strength gastric acid and successfully infect the gastric mucosa? And why can FSL effectively alleviate PU? It is really nonconvincing that at the initial stage *H. pylori* could infect gastric mucosa, which is in a highly acidic environment with pH 1.0−2.5.

Kinetic Analysis Reveals Bare *H. pylori* Is Unable To Directly Attack the Gastric Mucosa by Its Urease. Given in Table 4 are the values of K_m and V_{max} of HPUre 0.21 ± 0.06 mM (= 210 ± 60 μM) and 1200 ± 300 μM/min·mg (= 7.2 × 10^4 μM/h·-mg), respectively,[32] and the blood urea level S_{bu} = 2.5 × 10^3 μM; the gastric fluid volume production rate (dV/dt) = 0.104 L/h at pH 2.0 (C = 0.01 M HCl)[33] (Table 4).

Because the blood urea level (S_{bu}) is far in excess over the K_m value, HPUre always acts at its V_{max} provided that *H. pylori* cells

Table 4. Parameters Used To Calculate the Attack of Bare *H. pylori* on Gastric Mucosa from Gastric Lumen at pH 1.0−2.5

parameter	cited value	value used for calculation	ref
gastric production rate	2500 mL/24 h or 104 mL/h or 0.104 L/h	0.104 L/h	Harper et al.[33]
pH of gastric fluid	1.0−2.5	2.0 = 0.01 M HCl	Harper et al.[33]
K_m of urease	0.21 ± 0.06 mM	0.21 mM or 210 μM	Gang et al.[32]
V_{max} of urease	1200 ± 300 μM/min·mg	7.2×10^4 μM/h·mg	Gang et al.[32]
blood urea nitrogen (BUN)	8−20 mg/dL or 2.86−7.14 mmol/L	140 mg/L or 5 mM	Harper et al.[33]
blood urea	(taking the median value from BUN to calculate)	600 mg/L or 2.5×10^3 μM	Harper et al.[33]
E. coli cell			Wikipedia
size	l = 2 μm		
	w = 0.5 μm;		
volume	0.6−0.7 μm^3		
wet mass	≈1 pg		
dry wet mass, DCM	≈0.2 pg DCM		
cell count	10^9 cells/g wet mass		
total protein	assume = 40% DCM (0.08 pg)		
soluble protein	assume = 15% DCM (0.03 pg)		
H. pylori			
size	l = 3−4 μm	l = 4	
	w = 0.5−1 μm	w = 0.5	
volume	1.2−1.4 μm^3		calculated by taking *E. coli* as the reference database
wet mass	≈2 pg		
dry wet mass, DCM	≈0.4 pg DCM		
cell count	5×10^8 cells/g wet mass		
total protein	assume = 40% DCM (0.16 pg)		
soluble protein	assume = 15% DCM (0.06 pg)	15% (0.06 pg)/cell	

Table 5. Percent Viability of *H. pylori* in the Artificial Gastric Fluid as a Function of Chyme Particle Size and Contact Time[a]

contact time (min)	particle size of chymes (% viability)					
	intact chyme	dispersed chyme	80 mesh	60 mesh	40 mesh	20 mesh
0	100 ± 0.1 c,C	92.3 ± 3.1 a,B	92.5 ± 2.3 a,C	92.9 ± 2.5 a,C	93.4 ± 2.3 a,D	95.1 ± 3.1 b,D
20	100 ± 0.1 f,C	4.2 ± 1.5 a,A	31.4 ± 2.1 b,B	43.3 ± 3.6 c,B	57.2 ± 5.2 d,C	82.6 ± 4.2 e,C
40	96.2 ± 2.2 e,B	0.0	14.5 ± 2.2 a,A	18.5 ± 2.4 b,A	21.3 ± 3.3 c,B	28.6 ± 3.2 d,B
60	92.3 ± 1.8 c,A	0.0	0.0	0.0	5.3 ± 4.2 a,A	20.5 ± 4.2 b,A

[a] The artificial gastric fluid (AGF) was 1 M HCl (pH 2.4 ± 0.2) containing 4 mg of pepsin (Sigma-Aldrich, USA) per 100 mL of AGF. Data are expressed as the mean ± SD obtained from triplicate samples (n = 3). Different lower case letters in the same row indicate significant difference from each other ($p < 0.05$ or $p < 0.01$). Different upper case letters in the same column indicate significant difference from each other ($p < 0.05$ or $p < 0.01$).

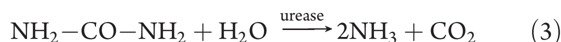
have successfully colonized the gastric mucosa. In addition, the gastric fluid production rate R_{gf} is

$$R_{gf} = C(dV/dt) = 0.01\ \mathrm{M} \times 0.104\ \mathrm{L/h} \quad (1)$$

$$= 1.04 \times 10^3\ \mu\mathrm{M/h} \quad (2)$$

Accordingly, the amount of ammonia required to neutralize the gastric fluid at pH 2.0 to end point pH 7.0 for optimum growth of *H. pylori* should correspondingly match the same production rate, 1.04×10^3 μM/h (Table 4).

Stoichiometrically, urease decomposes 1 mol of urea to give 2 mol of ammonia (eq 3).



$$NH_2{-}CO{-}NH_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2 \quad (3)$$

For urea decomposition, the decomposition rate (R_u) in the gastric fluid macroenvironment needs only half the value of the gastric fluid production rate (R_{gf})

$$R_u = R_{gf}/2 = (1.04 \times 10^3\ \mu\mathrm{M/h})/2 = 5.2 \times 10^2\ \mathrm{M/h} \quad (4)$$

Apparently, to suppress the gastric acidity, the minimum amount of enzyme urease protein (W_{ure}) required would be

$$W_{ure} = R_u/V_{max}$$

$$[5.2 \times 10^2\ \mu\mathrm{M/h}]\}/[7.2 \times 10^4\ \mu\mathrm{M/h \cdot mg}] = 7.2 \times 10^{-3}\ \mathrm{mg} \quad (5)$$

$$= 7.2 \times 10^6\ \mathrm{pg} \quad (6)$$

As mentioned, the *H. pylori* urea protein constitutes almost 6% of the soluble proteins,[30] which is approximately 0.06 pg/cell (Table 4), giving the urease protein of *H. pylori*.

$$P_{urease} = 0.06\ \mathrm{pg/cell} \times 0.06 = 3.6 \times 10^{-3}\ \mathrm{pg/cell} \quad (7)$$

Table 6. Hypothesized Biological Effect of FSL To Eliminate *H. pylori* in Vivo in GPU Victims

stage	gastric status	*H. pylori*(HP) status	host status	role of FSL
normal uninfected stage	normal acidity (pH 1.0−2.5), normal mucosal thickness, and parietal cell population	although contaminated in foods, incapable of penetrating the gastric mucosa (optimum survival pH range 5.0−8.0)	normal gastric physiology and biochemistry (blood pH 7.25−7.45; gastric pH 1.0−2.5)	nutrition supplement, hypoglyceridemic, hypocholesterolemic, antioxidant
preinfection stage	hyperacid, loss of parietal cells and mucous cells; thinner mucosa	a minimum critical population of HP hidden in a well-emulsified and buffered chyme	high stress both physically and mentally, malnutrition due to insufficient protein uptake	nutrition supplement, hypoglyceridemic, hypocholesterolemic, antioxidant
initial infection stage	hyperacid, very thin mucosal lining	HP in chyme attaches to mucosal surface, triggering urease (URE) to retard the acidic macroenvironment nearby mucosa; on penetrating into mucosa, proton pump (PP) is activated to neutralize blood pH and create an acidic microenvironment	appetite loss, nausea, simultaneously, suppressed immune system	nutrition supplement, buffering to retard the acidification of microenvironment created by small pioneer population of HP; triggering host immune system; anti-inflammatory
colonization stage	persistent gastric inflammation	colonized in mucosa, destroying parietal and mucous cell with aid of PP; migrating to gastric epithelium by help of PP with pH maintained at 5.0−7.0	stomachache, nausea, inflammation, no appetite, dyspepsia	buffering to retard the acidification of microenvironment of medium population of HP, activating host immune system, anti-inflammatory
epithelial invading stage	epithelial erosion; gastric hemorrhage	invading gastric epithelial macroenvironment with the help of PP and URE to maintain pH at 6.0−7.0 for optimum growth	inflammation, dyspepsia, gastritis, malnutrition, dystrophia, dyspepsodynia	buffering to destroy acidic microenvironment of huge population of HP; anti-inflammatory; lowering ROS and stress
gastropeptic ulcer (GPU) stage	gastric hemorrhage, gastroalbuminoorrhea	huge population existing in stomach, causing stomach erosion and ulcer; breath test positive	ulcer, nausea; ammonia breath	buffering; lowering ROS and stress, anti-inflammatory

Thus, the initial colonization in the gastric mucosa at pH 7.0 requires a population of bare *H. pylori*.

$$N_{\mathrm{Hp}} = 7.2 \times 10^{6}\ \mathrm{pg}/3.6 \times 10^{-3}\ \mathrm{pg/cell} = 2.0 \times 10^{9}\ \mathrm{cells} \quad (8)$$

Imagine that such a huge number of bare *H. pylori* cells should have been already existing previously and sticking together immediately before colonization, a situation unlikely achievable in the extremely high strength acidic gastric lumen fluid.

As mentioned, *H. pylori* can survive within pH 5−7 but is unable to divide at pH ≤6.0. At pH ≤3.0 or ≥9, its flagella proteins are completely inactivated.[27] Considering the initial successful attack and attachment on the gastric mucosa would be the major determinant factor of *H. pylori* infection, we hypothesize herein the "Microenvironmental Chyme Protection Theory". Principally, there must be a "well-buffered chyme shelter" protecting *H. pylori* to facilitate its initial attack onto the gastric mucosa. Only under such a circumstance can *H. pylori* have the least chance to survive; HPU is likely acting as a protector at the very early stage of colonization against the extremely tough extracellular macroenvironment (here we designate it to be the infinitely small junction between the chyme and gastric mucosa surrounded by gastric fluid having pH 1.0−2.5) (Figure 2). Conversely, PP acts as both a protective and an invasive tool in the microenvironment once it has colonized a focus in the gastric mucosa (Figure 2). To emphasize, the initial invasive power of *H. pylori* would not depend on the neutralization power of HPU excreted by bare *H. pylori* cells. Instead, the flagella penetration

and the neutralization of blood pH by the action of PP are acting as the dominant attacking powers. After penetrating into the gastric mucosa with the help of energetic flagella, PP is more activated to sustain a slightly more acidic microenvironment than blood within gastric mucosa to accommodate the survival and proliferation of *H. pylori*. As mentioned, a pH range of 6.0−7.0 is optimum for its growth[28] (Figure 2). Under such a condition with pH ≤7.0, a diversity of host immune systems simultaneously can be partially or completely suppressed. Moreover, blood pH is 7.25−7.45.[33] In essence, PP may multifunctionally act as an invasive factor, a protector, as well as an immunity suppressor by attenuating and inactivating host immune system by lowering the blood pH. Evidently, colonization of *H. pylori* in the gastric mucosa needs a close coordination of both PP and HPU (Figure 2). PP acidifies, whereas HPU alkalinizes, the environmental fluid pH, operating as a well compromised "two-point pH modulator" and creating a focus maintained at pH 6.0−7.0. Supposedly, FSL intervenes and destroys such a well-controlled microenvironment. To verify this hypothesis, we performed the chyme-shelter test to simulate the infection path of *H. pylori*. We prepared *H. pylori*-contaminated chymes of different particle sizes having *H. pylori* at a density of 2×10^5 bacteria cells/mL. The artificial gastric fluid with pH 2.4 ± 0.2 and at 37 °C was used to mimic the gastric fluid attack on *H. pylori* during the infection. The sheltering effect was prominently perceived. The intact, dispersed, and particulate (mesh sizes 80, 60, 40, and 20) chymes in artificial gastric fluid showed the cell viability of HP to be time- and size-dependent (Table 5). Larger chyme particles showed a more prevailing sheltering effect. For a chyme with a given particle size, the longer the contact time, the larger the population of *H. pylori* was killed. The cell viability was seen changing from 95.1 to 82.6%, to 28.6%, and to 20.5%, respectively, at time of contact from 0.0 to 20 min, to 40 min, and to 60 min (Table 5). With the contact time fixed at 20 min, the viability was 100, 4.2, 31.4, 43.3, 57.2, and 82.6%, respectively, for the intact, dispersed, and particulate chymes with mesh sizes 80, 60, 40, and 20. Implicitly, at the moment of attack on the gastric mucosa, HP has to be first protected by chymes acting as a "sheltered HP cluster".

Obviously, the prevalence of GPU depends on a diversity of factors including intrinsic and extrinsic factors. The intrinsic factors involve the physiological status of each individual (e.g., gastric hyperacidity, nutritional status, internal stress, immunostatus, etc.), and the extrinsic factors involve external stress, nutritional consumption, source of *H. pylori*, quantity of *H. pylori*, etc. (private communication from gastroenterologist Prof. Pan, Taipei Medical University, Taiwan). On the basis of these considerations, the nutraceutical and therapeutic role of FSL is proposed in Table 6. In brief, the prerequisite requires that the host must have been hyperacidic in stomach and simultaneously under a high level of stress. Under such a circumstance, the gastric mucosa will become thin enough to facilitate the penetration of *H. pylori* hidden and protected in chymes (Table 6). At this stage, a minimum critical population of *H. pylori* is required and, simultaneously, HPU is activated to create a favorable macroenvironment having pH 5.0−7.0 (Table 6). After having resided in the mucosa, PP of *H. pylori* is triggered to neutralize the alkaline bloodstream having a pH 7.25−7.45, maintaining the microenvironment at the optimum pH 6.0−7.0 for growth (Table 6). In brief, the effect of FSL to ameliorate PU can be very diverse and extremely complicated depending upon the pathological condition.

In summary, fermentation increased the isoflavonoids and essential amino acid contents by 2.5−17.3- and 1.5−4.0-fold in FSL. Kinetic analysis indicated that an effective infection in gastric fluid having pH 2.4 ± 0.20 requires a single cluster of 2.0×10^9 bare cells for a single one-strike attack, which is unlikely to occur in reality. Chyme-shelter testing showed the cell viability of HP was time- and size-dependent. implicating the attack by a sheltered HP cluster is more likely. FSL was shown to be beneficial to PU; 1 g/20 mL tid eradicated HP in 82% of 37 volunteers with PU after 3 months of treatment. Simultaneously their plasma CD and TBARs contents were resumed ($p < 0.20$). Suggestively, the action mechanism of FSL may involve (i) elevating blood alkalinity and buffering capacity to destroy the acidic microenvironment created by PP, (ii) providing valuable bioactive anti-inflammatory and vasodilating peptides, (iii) increasing blood flow to enhance nutritional supply to facilitate recovery of normal mucosa, and (iv) suppressing the ROS and interfering with macrophage recognition on ox-LDL.

Conclusively, kinetic analysis and chyme-shelter testing have revealed that the direct infection by bare *H. pylori* on the gastric mucosa is unlikely to occur in reality. HP may sustain its growth and proliferation by a two-point pH modulator. Fermentation increases the isoflavonoid and essential amino acid contents. Administration of FSL resumes plasma CD and TBARs levels ($p < 0.05$). Thus, FSL is beneficial in eradicating HP in GPU patients ($p < 0.20$).

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