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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Replacement of bakery shortening with rice bran oil in the preparation of muffins

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Studies were carried out to replace bakery shortening with refined rice bran oil in the preparation of muffins. Physico-chemical properties of bakery shortening and rice bran oil were studied. Rice bran oil was found to have a higher content of the essential fatty acid linoleic acid (34.98%) as compared to that of bakery shortening (5.14%). Chemical composition of wheat flour used was also studied. Muffin samples were prepared by replacing bakery shortening with rice bran oil at 0, 25, 50, 75 and 100% levels. Muffins were examined for quality that is weight, volume and specific volume and organoleptic quality that is appearance, colour, texture, flavour and overall acceptability on a 9 point hedonic scale. Statistical analysis revealed that muffin making and organoleptic quality of muffins prepared after replacing rice bran oil at the 50% level or greater varied significantly which is desirable from that of control. Statistically significant variations were observed in the texture of muffins prepared with shortening alone from that prepared after replacing bakery shortening with rice bran oil at 50% level.

Key words: Muffin, organoleptic quality, rice bran oil, shortening, texture.

INTRODUCTION

Rice is one of the most important crops in the world in addition to wheat and corn. Rice is cultivated in over 100 countries around the world and is a staple food for about half of the world population. The total paddy production area is about 154 million hectare and the annual production of rice is about 594 million tons. Brown rice grains contain more nutritional components, such as dietary fiber, E and B vitamins and gamma aminobutyric acid (GABA), than ordinary milled rice grains. These bio-functional components exist mainly in the germ and bran layers that are removed by polishing or milling (Champagne et al., 2004).

Rice bran oil (RBO) is generally considered to be one of the highest quality vegetable oil in terms of its cooking quality, shelf life and fatty acid composition (Sayre and Sunders, 1990). Rice bran oil is obtained from the outer brown layer of rice. Generally rice bran contains 15 to 20% oil (Marshall and Wadsworth, 1994). It is extensively used in Japan, Korea, China, Taiwan and Thailand as a “Premium Edible Oil”. The oryzanol present in rice bran is reported to have functions similar to vitamin E in promoting growth, facilitating capillary growth in the skin, and improving blood circulation along with stimulating hormonal secretion (Luh et al., 1991). Rice bran oil is an excellent source of polyunsaturated fatty acids (PUFA) which are helpful in lowering cardiovascular risks. Rice bran oil lowered human blood Low-density lipoprotein (LDL) cholesterol more effectively than did sunflower while high-density lipoprotein (HDL) remain unchanged using corn and safflower oils (Suzuki and Oshima, 1962). Kirk and Sawyer (1999) investigated that refined oil in good

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condition has thiobarbi turic acid (TBA) values of 0.02-0.08 whereas crude oil or badly stored oils have 0.1-0.2. Furia (1968) reported a commercial test based on the reaction of 2- thiobarbituric acid with the oxidation products of fats and oils to form a red color. Joo et al. (2001) investigated effects of rice bran oil on the oxidative stability and nutritional properties of restructured beef roasts and concluded that addition of 2% rice bran oil (w/w) is effective in improving both oxidative stability and vitamin E levels of restructured beef roasts.

In the usual two-stage muffins method, the shortening and sugar are combined and mixed. During this step the air is dispersed into the solid phase. Then the eggs are incorporated, followed by the flour, liquids, and other ingredients. During the first creaming step, the plastic shortening entraps air bubbles. In the presence of an emulsifier such as 4% monoglyceride, these bubbles are divided into numerous small air cells by agitation. The shortening must be solid (so the bubbles do not escape), but also plastic so it can fold around each air pocket. This is best accomplished by a plastic shortening, crystallized in the beta prime (β^{l}) phase. If the shortening has trans-formed into the β phase, the large plates of solid fat are much less effective in entrapping the fat. A good short-ening for this type of cake batter production has the solid fat index profile of all-purpose shortening, containing added monoglyceride. The effects of both type and level of shortening on batter viscosity, specific gravity and shear force values of white cakes were studied by Matthews and Dawson (1983). As the level of shortening increased, specific gravity and shear force values decreased, while batter viscosity increased with increase in fat level upto 50%.

Muffins are less sweet than most cakes, and generally have a somewhat more open crumb structure (a close, fine grain is not desired). The shortening used is an all purpose (nonemulsified) type to avoid more raising in volume during baking. In some instances, a vegetable oil shortening is used, when an extremely open texture accompanied by a moist mouthfeel is desired. Shortening levels vary widely, from 18 to 35% based upon the amount of flour; tenderness of the finished product varies accordingly.

The baking industry is a developing market sector in India, which is growing in its volume. The people are becoming more conscious about health and nutrition. Foods that are convenient, with good taste, reasonably priced and carry a favorable nutritional image are in great demand. Among bakery products, especially muffins and cookies, fat and oil are one of the major ingredients. The functional and nutritional properties of RBO has appeared well suited to its usage as shortening in baked goods like muffins. Little research was found in the recent literature regarding the performance of vegetable oils in muffins.

MATERIALS AND METHODS

Raw materials

Flour, sugar, rice bran oil, salt and other ingredients for product preparation were procured from local market.

Chemical analysis of flour

Chemical characteristics of flour were analysed using standard procedures (AACC 2000).

Protein

Standard AACC (AACC, 2000) procedure given under 46-11 A was followed. Sample (weighed) was digested in Kjeldhal flask with digestion mixture (copper sulphate and potassium sulphate in 1:10 ratio) and concentrated H_2SO_4 (20 ml) till light green color and cooled. Ammonia released by distillation of digested sample with saturated NaOH (80 ml) was captured in 0.1 N HCl to calculate % nitrogen (N_2). The protein content was calculated as per cent N_2 x factor. The factor of 6.25 was used for calculation.

Ash

Standard AACC (AACC, 2000) procedure given under 08-01 was followed. Weighed sample (5 g) was charred on hot plate and incinerated in furnace at 550±10°C for 3 h. It was cooled, weighed and ash content was expressed as% ash.

Fat

Hydrogenated fat (Gagan brand, manufactured by Gagan Vanaspathi Ltd.) which had a melting point of 37°C was procured from the local market and used as shortening in the formulae for baking tests.

Refined rice bran oil

Refined Rice bran oil (Ricela) was procured from A.P. Solvex Ltd, Dhuri.

Leavening agent

Commercial baking powder (Weikfield Company) was used as leavening agent in muffin preparation.

Sugar

Powdered sugar was purchased from the local market for use in muffin preparation.

Salt

Salt used in muffin preparation was purchased from the local market.

Chemical analysis of rice bran oil and bakery shortening

Rice bran oil and bakery shortening were subjected to chemical analysis for the determination of colour, iodine value, saponification value, moisture, refractive index, peroxide value, free fatty acid value, specific gravity, smoke point and flash point according to methods given by AOAC (2000). Fatty acid profile of rice bran oil and bakery shortening was also studied according to methods given by AOAC (2000).

Table 1. Formula used for preparation of muffins.

Ingredient	Quantity (g)
Flour	100
Fat	57.13
Sugar	78.58
Salt	0.50
Baking Powder	2.9
Essence (Vanilla)	1.0
Eggs	100
Water	Required milliliter to get desired consistency of batter

Table 2. Different treatments used in the study.

Treatment	Normal shortening (%)	Rice bran oil (%)
T_1	100	-
T_2	75	25
T_3	50	50
T_4	25	75
T_5	-	100

T_1 = 100% normal shortening (NS); T_2 = 75% NS + 25% Rice Bran Oil (RBO); T_3 = 50% NS + 50% RBO; T_4 = 25% NS + 75% RBO; T_5 = 100% RBO.

Muffin making

Various ingredients and their quantity used in the preparation of muffins are given in Table 1. For the muffins method, standard AACC method (AACC, 2000) was used. For muffin making, shortening was used according to ratios as mentioned in Table 2.

Evaluation of muffin making quality

The muffins prepared were analyzed for weight, volume by seed displacement method measured and specific volume.

Sensory evaluation

The muffin samples were evaluated for appearance, colour, texture, flavour and overall acceptability by a panel of six trained judges on a nine point hedonic scale.

Texture studies

Firmness of muffin samples was analyzed by Stable Microsystem Texture Analyzer, the settings used for test were given: Test, Compression test; Probe, Flat disc; Pre-test speed, 1 mm/s; Test speed, 1 mm/s; Post-test speed, 1 mm/s; Distance, 15 mm; Force, 60 kg.

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance according to the methods described by Steel et al. (1997).

RESULTS AND DISCUSSION

Chemical analysis of rice bran oil and bakery shortening

Data with regard to physico-chemical properties of bakery shortening and rice bran oil used in the study are presented in Table 3. Bakery shortening had colour 2R, 1.7Y, iodine value of 91.66, saponification value of 187.24, refractive index of 1.45, peroxide value of 0.92 meq/kg, free fatty acid value of 0.09%, moisture of 0.05%, specific gravity of 0.91, smoke point of 202.0°C and flash point of 271°C. Rice bran oil had colour 2.2R, 2Y, iodine value of 103.68, saponification value of 184.55, refractive index of 1.47, peroxide value of 0.88 meq/kg, free fatty acid value of 0.07%, moisture of 0.04%, specific gravity of 0.912, smoke point of 213° C and flash point of 280° C. Earlier Perzybylski and Mag (2002) studied the composition, properties and uses of vegetable oils used in food technology and reported similar results. Rice bran oil was found to be having a much higher content of linoleic acid (34.98) when compared to that of bakery shortening (5.14) as shown in Table 4.

Effect of different treatments on muffin making quality

Muffin volume and specific volume values were found to be more for muffins prepared after replacing bakery shortening with rice bran oil at 25, 50, 75 and 100% levels, in comparison to control (Table 5 and Figure 1). Maximum value for volume was observed in muffins prepared with 100% replacement of bakery shortening (170.00 cc) whereas minimum value for the same was observed in control (151.67 cc). Maximum value for specific volume was observed in muffins prepared with 100% replacement of bakery shortening (3.20 cc/g) whereas minimum value for the same was observed in control (2.78 cc/g). No significant differences were observed among control and different replacement levels in the values for weight. Earlier, Kamran et al. (2005) studied the development of improved quality of baked products by replacing bakery

Table 3. Physico-chemical properties of bakery shortening and rice bran oil.

Property	Bakery shortening	Rice bran oil
Colour (1.25" Lovibond red)	2R 1.7Y	2.2R 2Y
Iodine value	91.66	103.68
Saponification value	187.24	184.55
Refractive Index	1.45	1.47
Peroxide Value (meq/kg)	0.92	0.88
Free Fatty Acid (as% Oleic acid)	0.09	0.05
Moisture (%)	0.05	0.03
Specific gravity	0.91	0.91
Smoke point (°C)	202.00	213.00
Flash point (°C)	271.00	280.00

Each value is a mean of three observations.

Table 4. Fatty acid composition of bakery shortening and rice bran oil (g/100 g).

Sample	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidonic acid	Erucic acid
Bakery shortening	38.07	4.53	51.56	5.14	-	1.00	-
Rice bran oil	18.80	1.83	42.88	34.98	1.27	0.50	-

Each value is a mean of three observations.

Table 5. Effect of Different Levels of Rice Bran Oil on Muffin Making Quality.

Treatment	Weight (g)	Volume (cc)	Specific volume (cc/g)
T_1	54.50	151.67	2.78
T_2	54.00	155.00	2.87
T_3	55.17	166.67	3.03
T_4	54.17	163.33	3.02
T_5	53.17	170.00	3.20
CD (0.05)	NS	5.75	0.23

shortening with rice bran oil at 25, 50, 75 and 100% levels and reported similar results.

Effect of different treatments on the sensory quality of muffins

Muffins prepared after replacing bakery shortening with rice bran oil at 50% level were awarded higher scores for appearance in comparison to control. Maximum scores for texture were awarded to control muffins prepared with 100% bakery shortening (7.33) that is more tougher the muffins whereas minimum score was awarded to muffins prepared with 100% replacement of bakery shortening (6.17) that is more tender muffins. Overall acceptability of control muffins was best (7.96) in comparison to other levels of replacement (Table 6). Earlier, Kamran et al. (2005) studied the development of improved quality of baked products by replacing bakery shortening with rice bran oil at 25, 50, 75 and 100% levels and reported similar results.

Texture analysis of muffins

Control and muffins prepared after replacing bakery shortening with refined rice bran oils at 25, 50, 75 and 100% replacement levels were evaluated for firmness by texture analyser. Force required to compress (g) was measured. Significant variations were observed in firmness of muffins as shown in Table 7. Force required to compress the muffins was minimum (176.56 g) in case of muffins prepared by replacing bakery shortening with rice bran at 100% replacement level and maximum (186.26 g) in control muffins prepared with 100% bakery shortening.

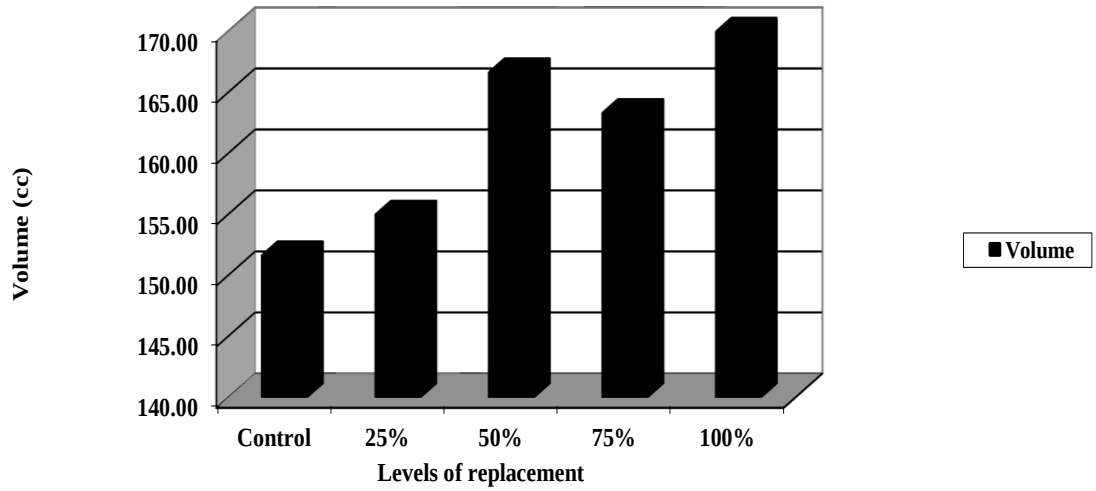

Figure 1. Effect of different levels of rice bran oil on the volume of muffins.

Table 6. Effect of different levels of rice bran oil on sensory quality of muffins.

Treatment	Appearance	Colour	Texture	Flavour	Overall acceptability
T_1	7.67	7.33	7.33	8.33	7.96
T_2	7.67	7.83	7.00	8.33	7.88
T_3	8.00	8.83	6.67	8.50	7.83
T_4	7.67	8.00	6.50	8.50	7.92
T_5	7.50	7.83	6.17	8.33	7.88
CD (0.05)	0.38	0.88	0.94	0.80	NS

Table 7. Effect of different levels of rice bran oil on the texture of muffins.

Treatment	Force (g)
T_1	186.26
T_2	185.76
T_3	183.51
T_4	181.33
T_5	178.46
CD (0.05)	0.001

Conclusion

It is concluded that bakery shortening can be successfully replaced with refined rice bran oil upto 50% level of replacement in the preparation of muffins with improvement in quality (functional and organoleptic) of the product. At 50% level of replacement, rice bran oil proved to be better than normal bakery shortening in the preparation of muffins as far as quality (functional and organoleptic) of the product was concerned. Hardness/crispness of the muffins decreased with increase in the level of replacement of bakery shortening with rice bran oil but the texture of muffins was found to be fairly acceptable upto 50% level of replacement of bakery shortening with rice bran oil.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

AACC (2000). Approved Laboratory Methods. 8th edn. American Association of Cereal Chemists St Paul, Minnesota.

AOAC (2000). Official Methods of Analysis. 16th edn. The Association of Official Analytical Chemists, Washington DC, USA.

Champagne ET, Wood DF, Juliano BO, Bechtel DB (2004). The rice grain and its gross composition. In: Champagne ET (ed) Rice. AACC, St. Paul, USA. pp. 77-107.

Furia TS (1968). The Hand Book of Food Additives. Ohio: The Chemical Rubber Co.

Joo SK, Godber JS, King JM, Prinyawiwatkul W (2001). Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran in an aqueous model system. J. Am. Oil Chem. Soc. 78:685-689.

Kamran S, Masood SB, Faqir MA, Muhammad N (2005). Improved Quality of Baked Products By Rice Bran Oil. M Sc. Thesis, Institute of Food Science & Technology, University of Agriculture Faisalabad, Pakistan.

Kirk RS, Sawyer R (1991). Pearson's Composition and Analysis of Foods. 9th edition. Harlow: AWL.

Luh BS, Barber S, Benedito de Barber C (1991) Rice bran: chemistry and technology, rice production and utilization. In: Luh BS. (ed.). New York: Van Nostrand Reinhold. pp. 313.
Marshall WE, Wadsworth JI (1994). Rice science and technology. New York: Marcel Dekker, Inc.
Matthews RH, Dawson EH (1983). Performance of fats and oils in pasteries and biscuits. Cereal Chem. 40:291.
Perzybylski R, Mag T (2002). Canola/rapeseed oil. In: Gunstone DF (ed) Vegetable oils in food technology: Composition, properties and uses. Blackwell Publishing Ltd USA. pp. 98-127.
Sayre RN, Saunders RM (1990). Rice bran and rice bran oil. Lipid Technol. 2:72.
Steel RGD, Torrie JH, Dickey D (1997). Principles and Procedures of Statistics. A biometrical approach. 3rd ed. McGraw Hill Book Co. Inc., New York.
Suzuki S, Oshima S (1962). Influence of blending oils on human serum cholesterol-rice bran oil, safflower and sunflower oil. J. Nutr. 28:194.

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Full Length Research Paper

Safety evaluation for multispecies probiotics in a 28-day feeding study in Sprague-Dawley rats

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This experiment evaluated the 28-day feeding study in rats after they were continuously fed with multi-species probiotics dosages in the low-dosage, medium-dosage, and high-dosage groups: 2,000 mg/kg body weight (B.W.), 4,000 mg/kg B.W., and 6,000 mg/kg B.W., respectively, for 28 days as a reference for the safety of its repeated usage. Each group consisted of 10 male and 10 female Sprague-Dawley (SD) rats and the test substance was administered for 28 continuous days. After the experiment, the rats were sacrificed, and their blood and viscera were collected for haematological analysis, serum bio-chemical analysis, and pathological examination. The test results showed that, during the experiment, none of the tested rats exhibited abnormal clinical symptoms; the rats in all dosage groups gained weight normally. At the end of the test, the results of urological testing, haematological testing, and serum biochemical testing revealed no significant differences between the dosage groups and the control group. The results of the pathological dissection, the macroscopic inspection of pathological changes, and the histopathological inspection revealed no significant pathological changes related to the tested substance in the dosage groups and the control group.

Key words: Probiotics, Sprague-Dawley rat, haematological analysis, serum biochemical analysis, pathological examination.

INTRODUCTION

Traditional dairy strains of lactic acid bacteria (LAB) are commonly regarded as safe because of their long history of use and have been given a “generally recognised as safe” (GRAS) status (Donohue, 2006). Probiotics have been generally defined as viable microorganisms which could confer a beneficial health effect on the host when administered in adequate amounts (Fuller, 1989). In order to demonstrate the efficacy of probiotics in improving human health, safety characteristics must be taken into consideration. However, new isolate-specific species or strains of probiotics, and novel probiotics cannot be assumed to share the historical safety of traditional strains (Salminen et al., 1998). Prior to incorporating new strains into products, their efficacy should be carefully assessed, and a case-by-case evaluation should be conducted to determine whether they share the safety status of traditional food-grade organisms (Salminen et al., 1998).

Different aspects of the safety of probiotics can be studied

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using *in vitro* and *in vivo* methods, including the Ames test, animal models, and human subjects (Salminen et al., 1998). Many countries, including those in the European Community, are currently developing more de-tailed guidelines with respect to regulations for novel and functional foods and related probiotic preparations. Conventional safety evaluation approaches, such as those for toxicological testing proposed by the Organisation for Economic Cooperation and Development (OECD), are appropriate as a first step in the evaluation of new probiotics (OECD, 2001). Therefore, a useful starting point could be a 28-day conventional rat-feeding study.

The available safety studies on LAB strains demonstrated that some *Lactobacillus* species, such as *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Enterococcus faecium*, have no oral toxicity to animals (Tsai et al., 2004a; Tsai et al., 2004b; Hirose et al., 2009; Szabo et al., 2011; Jones et al., 2012; Sulemankhil et al., 2012; Jia et al., 2011; Jia et al., 2013), but no research has evaluated the safety of multiple species or strains in the same product. In this study, the freeze dried powder of a novel multispecies probiotic mixture (PROBIO S-23) including *Lactobacillus rhamnosus* LCR177*, Bifidobacterium adolescentis* BA286*,* and *Pediococcus acidilactici* PA318, were isolated from pickled vegetables and human feces, respectively.

In a previous study, we suggested that exposure to multispecies probiotics did not cause mutagenicity, such as did not provoke reverse mutation, chromosomal aberrations, and micronucleated reticulocytes in bacteria, mammalian cells, and mouse peripheral blood, respectively (Chiu et al., 2013). The aim of this study was to evaluate the safety of multispecies probiotics based on the methods suggested for the safety evaluation of novel probiotics. We confirmed the safety of the multispecies mixed powder product by a 28-day feeding study using Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Test substance

Stock culture collections were maintained at -80°C in Lactobacilli MRS broth (DIFCO, Detroit, Michigan, USA) containing 25% glycerol. Cells were propagated twice in Lactobacilli MRS broth containing 0.05% L-cysteine by incubation at 37°C for 20 h. Bacterial counts were determined by plating serial dilutions of the culture on MRS agar. Plates were incubated at 37°C for 48 h anaerobically. The test substance “PROBIO S-23” is a mixture including *L. rhamnosus* LCR177*, B. adolescentis* BA286, and *P. acidilactici* PA318, Isomalto-oligosaccharide, xylo- oligosaccharide, Fibersol 2, and the final total lactic acid bacteria cells was $5.0x10^{10}$ CFU/g.

Animals and experimental design

Eighty Sprague-Dawley (SD) rats (40 males and 40 females) with ages of 7 to 8 weeks were used. The animals were obtained from the Experimental Animal Centre of the National Yangming University (Taipei, Taiwan). The housing temperature was 22 ± 4°C, with a relative humidity of 40-70% and alternate light and darkness of 12 h each. The female and male rats were kept separately, with two animals per cage. The animals were fed Laboratory Autoclavable Rodent Diet® 5010 (PMI Nutrition International, USA). Sterile plastic water bottles were placed in the breeding cages, and the animals were not restricted (ad libitum) from drinking reverse osmosis (RO) water.

The animals were processed in accordance with the Guide for the Care and Use of Laboratory Animals. All procedures were in accordance with requirements of the Institutional Animal Care and Use Committees (IACUC) of the Super Laboratory Co., Ltd (IACUC NO.: 100-9C, New Taipei City, Taiwan). This study tested control (RO water), low-dosage, medium-dosage, and high-dosage groups. The tested substance dosages in the low, medium, and high-dosage groups were 2,000 mg/kg body weight (B.W.), 4,000 mg/kg B.W., and 6,000 mg/kg B.W., respectively. With a sterile orogastric tube, three of the four groups were orally inoculated with the tested substance in RO water at three different doses.

During the testing period, clinical observation was conducted daily, and abnormal clinical symptoms or deaths after administration of the tested substance were recorded. All rats exhibiting abnormal clinical symptoms or death were recorded on log sheets for individual-animal clinical observation. The eye examinations first employed macroscopic observation to find any external abnormalities and then utilised an ophthalmoscope to examine the eyes’ internal structures. The eyes of all animals were examined before the administration of the probiotics and 1 day before being sacrificed at the end of testing. The body weight (B.W.) of each test animal was measured before the administration and once a week during the study.

Urinalysis

On the day before sacrifice, the animals were placed in a metabolism cage for 16 h, and their urine was collected. Specific gravity (SG), color, protein, urobilinogen, pH, ketone, bilirubin, glucose, nitrite, and occult blood were determined with a semi-quantitative biochemical urinalysis system (Urisys 2400, Roche, Basel, Switzerland). White blood cells (WBCs), red blood cells (RBCs), epithelial cells (EPs), crystals, and microorganisms were observed with microscopy.

Haematology

Before being sacrificed, the test animals were fasted overnight and anesthetised with carbon dioxide (CO_2). Blood samples were collected from the heart. The blood was placed in an anticoagulation tube containing ethylenediaminetetraacetic acid (EDTA), mixed evenly at room temperature, and analysed with an automatic blood analyser (Gen.S™, Beckman, USA). Hematocrit, hemoglobin, RBCs, WBCs, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte, neutrophil, and monocyte were determined. Another anticoagulation tube containing sodium citrate was used to collect the blood, and a blood prothrombin analyser (CA-1500, Sysmex) was employed to test the prothrombin time.

Blood biochemistry

The blood was kept at room temperature for solidification and then centrifuged to isolate the serum. The serum was analysed using a serum biochemistry analyser (LX®-20, Beckman). Plasma concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GT), albumin, total protein, total bilirubin,

Table 1. The body weight of SD rats orally administrated PROBIO S-23 at three different dose levels for 28 days.

Time	Body weight (g)			
	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
Day 0	264.5±9.4[a]	264.8±9.5	264.5±10.0	264.1±9.1
Week 1	306.0±11.5	306.2±16.0	309.2±14.2	299.5±17.7
Week 2	347.8±18.3	344.3±19.2	345.1±15.1	340.9±14.2
Week 3	365.2±19.1	365.2±19.9	366.8±18.9	362.6±16.7
Week 4	389.6±21.4	392.9±25.8	394.5±19.4	381.6±19.2
After fasting	360.8±21.6	365.4±24.7	365.1±20.8	353.4±19.0
Female				
Day 0	162.0±7.7	162.2±7.7	162.2±7.6	162.1±7.8
Week 1	191.1±12.3	189.7±10.4	188.3±10.7	189.2±9.9
Week 2	211.4±13.8	208.9±9.7	207.4±10.6	209.1±13.9
Week 3	222.7±19.8	225.1±13.9	223.6±15.4	228.3±17.4
Week 4	241.8±18.3	244.1±16.3	240.0±15.7	244.1±21.4
After fasting	220.8±18.3	222.1±15.1	220.5±14.0	223.3±19.3

[a]Values are mean±S.D.

creatinine, blood urea nitrogen (BUN), glucose, cholesterol, triglyceride, phosphorus, calcium, chloride, potassium, and sodium were determined.

Histopathology

The following main organs (brain, heart, kidneys, liver, spleen, adrenal glands, and testes or ovaries) were removed from all surviving rats. After removal of the peripheral adipose tissue, the absolute weights of the above-mentioned organs were weighed and recorded. Next, the ratio of organ weight to B.W. (on day 29 of the experiment) was calculated for all organs. The ratio of organ weight to B.W. (%) = [organ weight (g) / B.W. (g)] x 100.

The brain, heart, kidneys, liver, spleen, adrenal glands, testes, and ovaries from all experimental groups were removed and fixed in 10% neutral formalin for preservation (the testes were fixed in modified Davidson`s solution for 24 h and then fixed in 10% neutral formalin for preservation). After fixation in 10% neutral formalin solution, sections were cut from the adrenal glands, brain, heart, kidneys, liver, spleen, and testes or ovaries of the control group and the high-dosage (6,000 mg/kg/day) group of 40 rats and examined for histopathology. The formalin-solution-fixed adrenal glands, brain, heart, kidneys, liver, spleen, and testes or ovaries were coarsely repaired and were subsequently processed via dehydration, clarification, paraffin infusion, and embedding steps to prepare paraffin tissue blocks. A microtome (Leica RM 2145, Nussloch, Germany) was then used to slice the blocks into 5-μm-thick tissue slices. The slices were stained with haematoxylin & eosin (H&E) and were observed under an optical microscope (Opticphot-2, Nikon, Tokyo, Japan) for histopathological changes in all organs. If any organ and/or tissue in the high-dosage group displayed pathology related to the tested substance, then that organ and/or tissue in the medium-dosage group (4,000 mg/kg/day) and low-dosage group (2,000 mg/kg/day) of animals had to undergo histopathological inspections.

Statistics

The experimental data were expressed as the mean and the standard deviation (S.D.). All data of the animals' B.W., food intake, organ weight, haematological analysis, and serum biochemical analysis were analysed by the Duncan test method of one-way analysis of variance (ANOVA) using the SPSS statistical software package. This method analysed the variance in the data among the different groups, and a probability (p) value less than 0.05 was taken as the standard for significance.

RESULTS AND DISCUSSION

Mortality rate, observation of clinical symptoms, B.W., food intake, and eye examination

During the experiment, no rats in any experimental group or in the control group died. During the experiment, two daily inspections of all experimental rat groups by the veterinarians revealed that no rats in any dosage group or the control group exhibited any abnormal clinical symptoms. The mean B.W. values for the male rats and female rats in all dosage groups and in the control group are listed in Table 1. The experimental results indicated that, compared with the control group, the B.W. values for the rats in all dosage groups exhibited no significant difference (p>0.05), suggesting that the rats in all dosage groups were able to gain weight normally.

The mean food-intake values for the male rats during the experimental period are listed in Table 2. The experimental results indicated that the food-intake values of male rats in the high-dosage group were significantly lower than those in the control group (p<0.05) during the 1st week of the experiment; there was no significant difference in the remaining periods for any of the dosage groups in comparison with the control group. The mean food-intake values for female rats in the experimental period are also presented in Table 2. There was no

Table 2. The feed intake of SD rats of three different PROBIO S-23 doses groups and the control set among 28 days.

Time	Feed intake（g/rat/day）			
	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
Week 1	28.9±1.6[a]	27.8±1.6	27.9±1.3	25.2±2.5*
Week 2	32.2±1.6	31.7±1.6	31.1±2.5	29.7±0.8
Week 3	30.3±2.3	29.9±1.8	30.4±2.1	30.5±1.7
Week 4	29.5±1.8	28.9±3.1	26.8±0.8	26.6±0.6
Female				
Week 1	24.0±2.3	24.7±1.6	23.7±2.8	23.0±2.1
Week 2	23.8±1.5	23.7±0.7	24.1±1.1	22.7±2.2
Week 3	20.2±1.0	19.0±1.0	18.8±1.4	18.8±1.2
Week 4	17.5±1.0	17.8±0.4	16.8±1.3	16.3±1.6

[a]Values are means ± S.D (n=5, two rats in each cage). *Significantly different from control group at $p<0.05$.

significant difference ($p>0.05$) in the mean food-intake values for any of the dosage groups compared with those of the control group. Eye examinations by naked eye and an ophthalmoscope were performed before the initial administration of the tested substance and at the end of the test; no abnormalities were found.

Urinalysis

Results of the microscopic examination of the urine sediment were the number of EPs, RBCs, WBCs, and urinary crystals exhibited no significant differences between any of the dosage groups and the control group. Conventional urine tests revealed no significant differences between any of the dosage groups and the control group regarding the colour, glucose, bilirubin, ketobodies, specific gravity, pH value, protein, urobilinogen, and nitrite.

Haematological analysis

The haematological analysis results for the male rats demonstrated that, except for a significant rise ($p<0.05$) in the number of reticulocytes in the low-dosage and medium-dosage groups compared with the control group, there was no significant difference ($p>0.05$) between any of the dosage groups and the control group for the remaining test items (Table 3). Regarding the female rats, the experimental results showed no significant difference ($p>0.05$) between the dosage groups and the control group (Table 3).

Serum biochemistry analysis

The serum biochemistry analysis results for the male rats revealed no significant difference between any of the dosage groups and the control group (Table 4). Concerning the female rats, except for the significantly higher ($p<0.05$) serum alkaline phosphatase (ALP) concentration in the high-dosage group compared with the control group, there was no significant difference ($p>0.05$) between any of the dosage groups and the control group for the remaining items (Table 4).

Organ weight

At the end of the experiment, the surviving rats were euthanized, and their brains, livers, kidneys, spleens, hearts, adrenal glands, and testes or ovaries were extracted and weighed. The results revealed no significant difference in the absolute organ weight of the male rats or female rats in any of the dosage groups compared with those in the control group (Table 5). There was no significant difference ($p>0.05$) in the relative weight of the male rat organs between the dosage groups and the control group, except for a significant decrease ($p<0.05$) in the relative weight of the livers in the high-dosage group (Table 6). There was no significant difference ($p>0.05$) in the relative weight of the main organs in the female rats between any of the dosage groups and the control group (Table 6).

Histopathological interpretation

After the experiment, the results of the pathological dissection and macroscopic pathological examination of all groups demonstrated that no macroscopic pathology related to the tested substance was detected in the heart, lungs, liver, spleen, kidneys, digestive tract, brain, and geni-tourinary system (Figure 1).

Results of the nonspecific pathological interpretation, the heart of one male rat from the control group exhibited focal, minute, focal mononuclear inflammatory-cell infiltration, with an incidence of 1/10 among the male rats in the control group (Figure 2A). There was no positive correlation

Table 3. Haematological findings in rats treated orally with PROBIO S-23 for 28 days.

Parameter	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
WBC (10^3/μL)	11.8±5.0[a]	11.7±4.4	11.3±4.8	11.5±3.6
RBC (10^6/μL)	8.6±0.3	8.3±0.3	8.5±0.3	8.4±0.3
Hemoglobin (g/dL)	16.6±0.4	16.1±0.8	16.6±0.6	16.4±0.7
Hematocrit (%)	52.3±1.1	51.2±2.0	52.0±1.8	51.7±1.7
MCV (fL)	61.1±1.9	61.4±2.0	61.0±1.4	61.3±1.1
MCH (pg)	19.4±0.6	19.3±0.6	19.4±0.4	19.4±0.4
MCHC (g/dL)	31.7±0.5	31.4±0.6	31.9±0.5	31.7±0.5
Platelet (10^3/μL)	872.2±130.0	957.2±150.2	923.1±126.3	1016.6±196.6
Neutrophil (%)	13.9±5.7	13.1±3.5	14.5±3.7	12.5±4.1
Lymphocyte (%)	81.0±6.5	82.3±4.0	80.3±4.4	82.4±5.0
Monocyte (%)	3.8±0.9	3.5±1.4	3.9±1.3	3.8±1.1
Eosinophil (%)	1.2±0.6	0.9±0.4	1.2±0.3	1.2±0.4
Basophil (%)	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.1
Reticulocyte (%)	3.8±0.9	5.2±1.0*	4.9±0.8*	4.0±0.9
PT (s)	12.5±1.3	12.2±1.2	12.7±1.1	12.9±0.8
Female				
WBC (10^3/μL)	9.0±3.5	9.5±2.2	8.5±4.4	8.1±2.7
RBC (10^6/μL)	8.4±0.4	8.5±0.2	8.3±0.2	8.4±0.3
Hemoglobin (g/dL)	16.5±0.5	16.4±0.6	16.3±0.6	16.5±0.6
Hematocrit (%)	53.3±1.3	53.6±2.0	53.2±2.3	53.7±1.6
MCV (fL)	63.4±3.0	63.4±2.0	64.4±1.9	64.2±1.5
MCH (pg)	19.6±0.5	19.4±0.5	19.8±0.4	19.7±0.4
MCHC (g/dL)	30.9±0.8	30.6±0.5	30.7±0.5	30.6±0.6
Platelet (10^3/μL)	877.6±110.8	955.3±102.4	870.7±123.0	905.7±94.4
Neutrophil (%)	11.2±3.8	11.5±3.9	11.7±3.6	10.4±2.7
Lymphocyte (%)	83.6±3.7	83.1±4.8	82.6±4.1	83.7±3.5
Monocyte (%)	3.9±1.6	4.0±1.9	4.5±1.7	4.6±1.5
Eosinophil (%)	1.1±0.4	1.2±0.3	1.1±0.3	1.1±0.4
Basophil (%)	0.3±0.4	0.2±0.2	0.2±0.1	0.3±0.2
Reticulocyte (10^3/μL)	3.8±0.8	4.2±0.9	4.3±0.4	4.3±0.5
PT (s)	10.2±0.2	10.2±0.3	10.5±0.6	10.2±0.2

MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PT, Prothrombin time. a Values are means ± S.D. *Significantly different from control group at $p<0.05$.

of the tested substance to the occurrence of pathological changes in the female and male rats in the control group and the high-dosage group. In female rats from the control and high-dosage groups, the renal cortex and medulla exhibited a focal, minute-to-small, tubular mineral deposit, with an incidence of 7/10 for each group (Figure 2B). There was no positive correlation between the tested substance and the occurrence of pathological changes in the control and high-dosage groups of female rats. In female rats of the control and high-dosage groups, the liver exhibited multiple, minute-to-small fat-droplet infiltration, with an incidence of 4/10 and 7/10, respectively (Figure 2C). There was no positive correlation of the tested substance to the occurrence of pathological changes in the control and high-dosage groups.

In the previous study, the multispecies probiotic mixture showed no mutagenic potential that leads to bacteria reverse mutation, *in vitro* chromosome aberration, and micronucleated reticulocytes in mouse peripheral blood (Chiu et al., 2013). In this study, the 28-day administration of low, medium and high-dose mixed LAB strains did not produce any deaths or clinical signs of toxicity. Hepatomegaly and splenomegaly are usually indirect indicators of infection and invasion. Clinical chemical assays can be used to detect moderate to mild deficiency of nutrients or imbalances in nutrient metabolism, and these deficiencies

Table 4. Blood biochemistry in rats treated orally with PROBIO S-23 for 28 days.

Parameters	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
AST (U/L)	131.6±45.5[a]	124.8±18.9	119.1±14.9	120.6±13.8
ALT (U/L)	53.9±26.9	46.3±8.4	43.6±7.8	44.7±8.1
ALP (U/L)	125.6±25.0	148.1±32.2	153.9±43.3	149.0±15.5
T. bilirubin (µg/dL)	49.1±14.8	50.1±17.1	40.7±8.3	51.9±15.0
T. protein (g/dL)	6.7±0.3	6.7±0.4	6.6±0.3	6.6±0.3
Albumin (g/dL)	4.3±0.2	4.3±0.3	4.3±0.2	4.2±0.2
Globulin (g/dL)	2.4±0.1	2.4±0.2	2.4±0.2	2.4±0.1
BUN (mg/dL)	16.1±2.0	15.7±1.9	16.7±2.4	16.2±1.8
Creatinine (mg/dL)	0.40±0.04	0.36±0.07	0.37±0.07	0.36±0.06
Glucose (mg/dL)	181.0±37.0	167.9±26.3	175.0±44.8	168.2±31.7
Triglyceride (mg/dL)	28.0±3.3	28.0±4.2	30.8±8.1	28.3±8.2
Cholesterol (mg/dL)	58.8±16.5	66.1±13.6	61.5±16.8	53.9±13.9
Sodium (meq/L)	149.0±1.9	149.9±3.2	149.2±1.7	149.0±2.9
Potassium (meq/L)	7.6±0.7	7.5±1.1	7.4±0.8	7.7±0.6
Calcium (meq/L)	11.3±0.3	11.4±0.6	11.6±0.6	11.6±0.5
Chloride (meq/L)	99.3±1.5	100.6±3.0	100.0±2.2	100.4±1.6
Phosphorus (mg/dL)	12.3±0.9	12.6±1.2	12.3±0.7	12.3 ±1.0
Female				
AST (U/L)	174.4±149.4	142.7±59.0	153.9±28.8	141.9±32.9
ALT (U/L)	59.2±48.6	50.5±19.2	50.1±17.3	47.3±12.4
ALP (U/L)	108.0±20.6	117.4±23.6	122.2±33.8	146.2±36.2*
T. bilirubin (µg/dL)	59.5±17.6	64.1±18.6	61.3±14.5	55.2±21.5
T. protein (g/dL)	7.0±0.2	7.1±0.4	6.7±0.3	6.9±0.3
Albumin (g/dL)	4.5±0.2	4.6±0.2	4.4±0.2	4.5±0.2
Globulin (g/dL)	2.4±0.2	2.5±0.2	2.3±0.2	2.4±0.1
BUN (mg/dL)	17.4±2.4	15.4±2.1	15.5±3.9	15.7±3.0
Creatinine (mg/dL)	0.44±0.04	0.43±0.05	0.40±0.05	0.41±0.07
Glucose (mg/dL)	124.6±18.4	135.3±39.3	132.8±9.5	120.1±41.2
Triglyceride (mg/dL)	38.3±5.0	42.1±9.7	43.1±4.6	40.9±7.8
Cholesterol (mg/dL)	86.7±15.2	87.7±13.0	88.7±10.2	91.9±14.4
Sodium (meq/L)	146.8±1.2	148.0±1.1	147.6±1.1	147.6±1.3
Potassium (meq/L)	7.8±0.8	7.6±0.5	8.0±1.5	7.4±0.9
Calcium (meq/L)	11.8±0.3	11.9±0.4	11.9±0.3	11.9±0.3
Chloride (meq/L)	100.5±1.7	101.5±1.4	101.1±0.7	100.6±1.0
Phosphorus (mg/dL)	13.0±1.3	12.9±1.6	13.0±1.2	13.0±1.6

[a] Values are means Mean ± S.D. * Significantly different from control group at $p<0.05$.

are usually apparent before any clinical symptoms or changes in body weight (Swendseid et al., 1987). In this study, we did not find macroscopic changes in liver and spleen morphology of animals treated with test strains *in vivo*. Animals in these groups showed similar daily feed when compared with the control group. There was no significant difference in SGR between the treatment groups and the control group for rats ($p>0.05$). These results also suggest that the rats experienced no infections resulting from the 28-day treatment with multiple LAB strains.

Szabo et al. (2011) demonstrated the safety of *Lactobacillus pentosus* strain b240. Although statistical significance was shown for several parameters in this study, such as haematology, clinical chemistry or organ weights, among others, the authors hypothesised that none of these changes were attributable to treatment because they remained within the range of historical

Table 5. Absolute organ weight in SD rats treated orally with PROBIO S-23 at three different dose levels for 28 days.

Organ	Absolute organ weight (g)			
	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
Testes	2.99±0.12[a]	3.14±0.26	3.12±0.21	2.95±0.31
Spleen	0.68±0.10	0.71±0.14	0.63±0.07	0.65±0.09
Kidney	3.21±0.20	3.23±0.36	3.21±0.19	3.06±0.22
Adrenals	0.056±0.008	0.055±0.012	0.055±0.005	0.060±0.010
Liver	11.8±0.9	11.8±1.3	11.7±1.0	10.7±1.1
Heart	1.44±0.12	1.40±0.11	1.42±0.12	1.35±0.12
Brain	1.92±0.07	1.95±0.03	1.94±0.08	1.92±0.08
Female				
Ovaries	0.092±0.015	0.093±0.017	0.089±0.011	0.090±0.009
Spleen	0.50±0.05	0.49±0.08	0.54±0.06	0.54±0.09
Kidney	2.12±0.22	2.04±0.20	2.10±0.16	2.07±0.18
Adrenals	0.069±0.009	0.072±0.011	0.075±0.009	0.073±0.008
Liver	8.36±0.86	8.16±0.78	8.20±0.63	8.04±0.65
Heart	0.90±0.11	0.89±0.04	0.89±0.07	0.89±0.11
Brain	1.83±0.10	1.80±0.13	1.79±0.10	1.82±0.08

[a] Values are means mean ± S.D.

Table 6. Organ relative weight in SD rats treated orally with PROBIO S-23 at three different dose levels for 28 days.

Organ	Organ relative weight (g/ 100 g b.w.)			
	Control (0 mg/kg) (n=10)	Low dose (2,000 mg/kg) (n=10)	Medium dose (4,000 mg/kg) (n=10)	High dose (6,000 mg/kg) (n=10)
Male				
Testes	0.83±0.07[a]	0.86±0.10	0.86±0.07	0.83±0.07
Spleen	0.19±0.02	0.20±0.04	0.17±0.02	0.18±0.02
Kidney	0.89±0.05	0.88±0.08	0.88±0.07	0.87±0.05
Adrenals	0.016±0.003	0.015±0.003	0.015±0.001	0.017±0.002
Liver	3.28±0.13	3.23±0.20	3.21±0.18	3.02±0.23[*]
Heart	0.40±0.05	0.39±0.04	0.39±0.04	0.38±0.04
Brain	0.53±0.03	0.54±0.03	0.53±0.04	0.55±0.03
Female				
Ovaries	0.042±0.008	0.042±0.006	0.040±0.005	0.041±0.005
Spleen	0.23±0.02	0.22±0.03	0.24±0.02	0.24±0.03
Kidney	0.96±0.08	0.92±0.04	0.96±0.06	0.93±0.05
Adrenals	0.031±0.004	0.033±0.005	0.034±0.004	0.033±0.003
Liver	3.78±0.18	3.68±0.22	3.72±0.16	3.61±0.16
Heart	0.41±0.02	0.40±0.02	0.41±0.02	0.40±0.03
Brain	0.84±0.08	0.81±0.05	0.81±0.07	0.82±0.08

Relative weight = (organ weight/body weight) x 100; [a] Values are means Mean ± S.D. * Significantly different from control group at $p<0.05$.

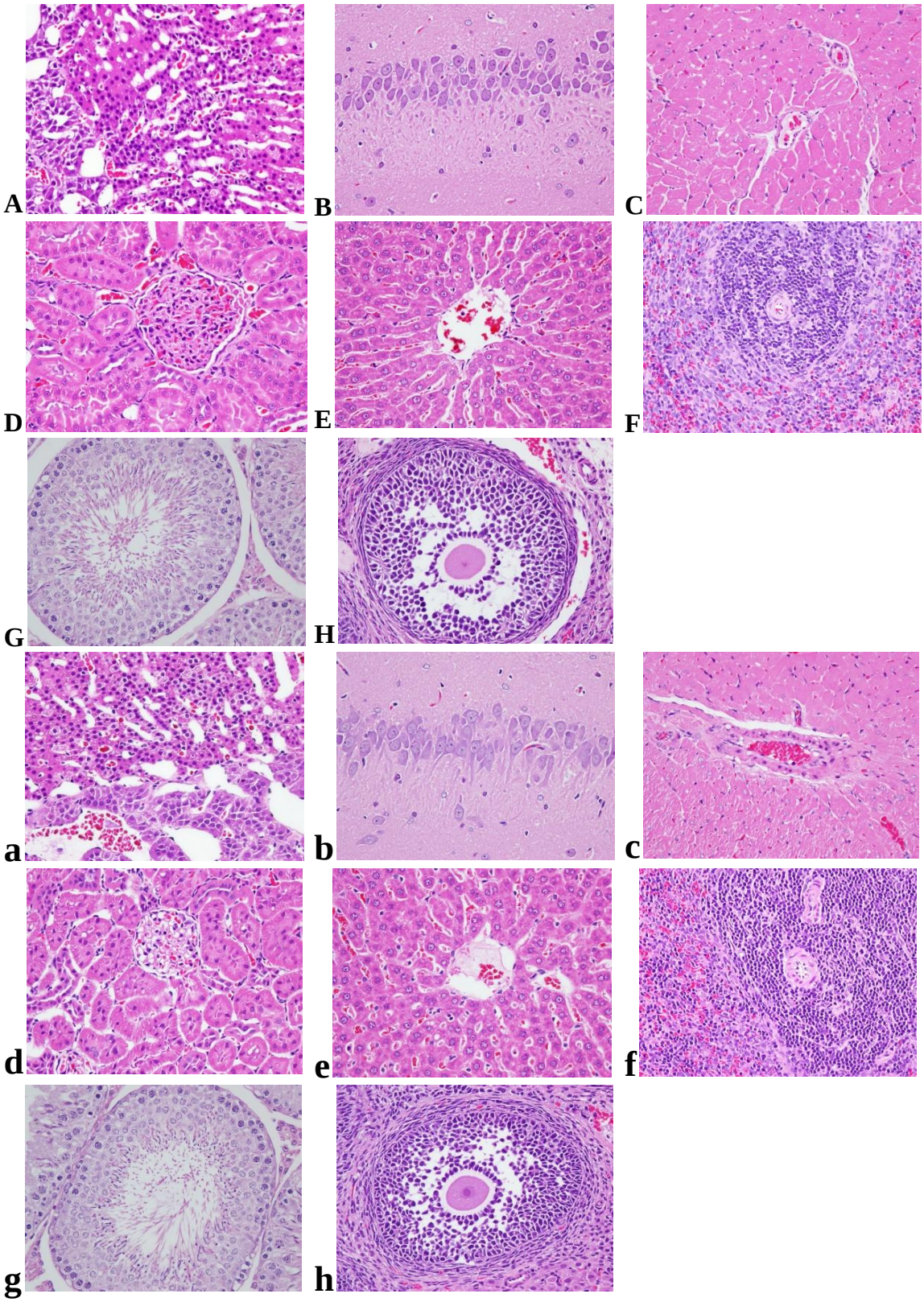

Figure 1. Photomicrographs of (A) adrenal glands, (B) brain, (C) heart, (D) kidneys, (E) liver, (F) spleen, (G) testes, and (H) ovaries histopathology from representative SD rats treated with RO water (control group), (a) adrenal glands, (b) brain, (c) heart, (d) kidneys, (e) liver, (f) spleen, (g) testes, and (h) ovaries histopathology from representative SD rats treated with PROBIO S-23 (6,000 mg/kg B.W, the high-dosage group) (Hematoxylin and eosin stain, 400x).

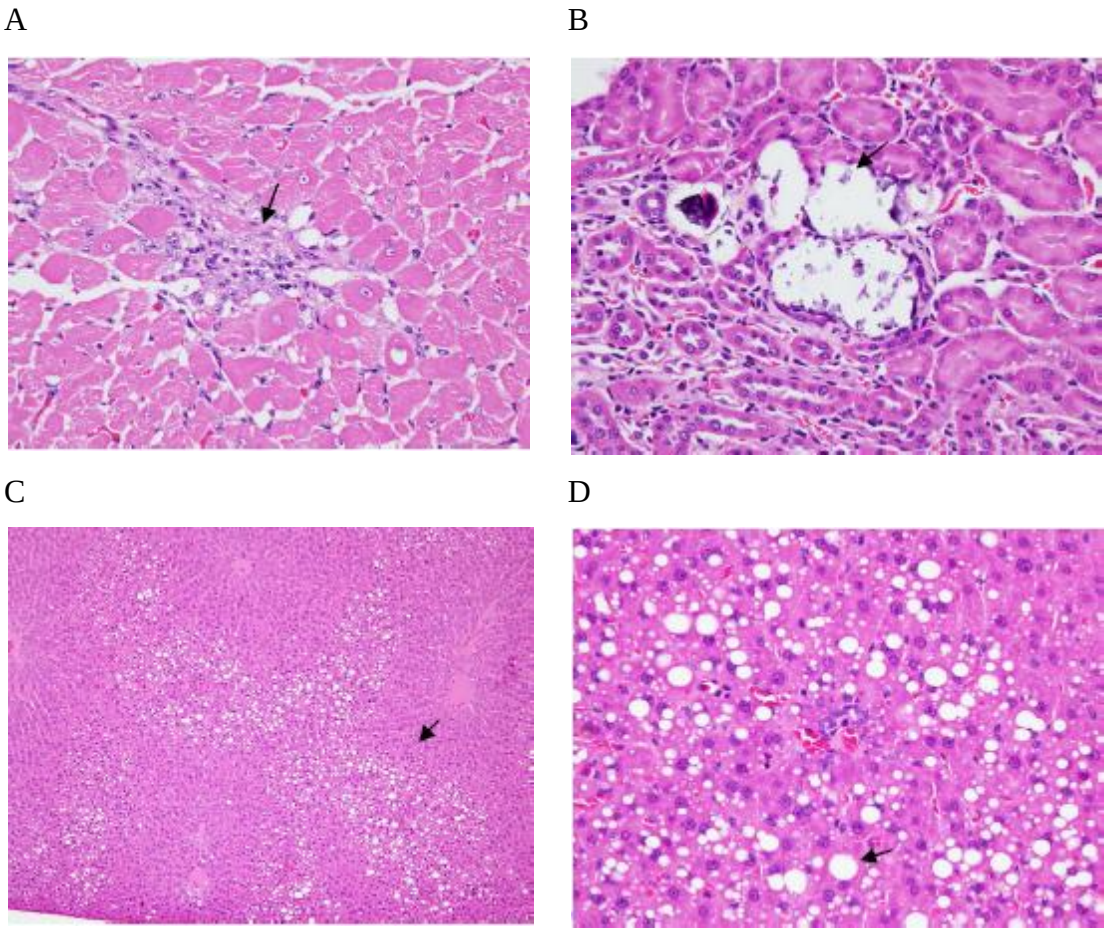

Figure 2. The nonspecific pathological interpretation of the organs in rats treated orally with purified water or PROBIO S-23 for 28 days. The heart of one male rat from the control group exhibited focal, minute, mononuclear inflammatory-cell infiltration (A, animal No. 05M, 400x). In female rats, the renal cortex and medulla exhibited a focal, minute-to-small, tubular mineral deposit (B, animal No. 04F, 400x). In female rats, the liver exhibited multiple, minute-to-small fat-droplet infiltration (C, 100x; D, 400x, animal No. 04F) (Hematoxylin and eosin stain).

controls, presented in only one sex, or noted only sporadically. In our study, some statistically significant results, such as the number of reticulocytes (male rats, the low and medium dose groups), serum alkaline phosphatase (female rats, the high dose group) and the relative weight of the livers (male rats, the high dose group), were observed as mentioned above.

Some rats exhibited focal, minute-to-small renal-tubular mineral deposits. The incidence of calcite deposition is high in SD, Wistar, and F344 female rats, which may be related to oestrogen (Reeves et al., 1993). Moreover, when the ratio of calcium (Ca^{++}) to phosphorus (P^{-}) in the feed is less than 1, magnesium (Mg^{++}) deficiency, chlorine (Cl^{-}) deficiency, or a high-pH urine can also induce a high incidence of calcite deposition (Schoenmakers et al., 1989). The calcite deposition in the renal pelvis can block the kidney tubules, which slows the speed of urination and thus leads to chronic progressive nephropathy, such as renal tubule enlargement and the accumulation of protein-like homogeneous exudates in the renal glomerulus, Bowman's capsule, and renal tubular cavity. However, long-term experiments on chronic toxicity have revealed no direct correlation between calcite deposition pathology and cancer (Rao, 2002; Owen and Heywood, 1986). In addition, the focal, minute mononuclear-cell infiltration in the heart of one male rat from the control group and the multiple, minute-to-small fat-droplet infiltrations in the hearts of female rats from the control and high-dosage groups were physiological phenomena.

Conclusions

Based on above-mentioned test results, the no-observed-adverse- effect level (NOAEL) for 28-day repeat dosages of PROBIO S-23 for rats was greater than 6,000 mg/kg/day. The margin of safety was greater than 80-fold safety factor. In Taiwan, the Food and Drug Administration

allows a 60-fold safety factor in the 28-day toxicity testing for natural extracts and Probiotic products. We conclude that consumption of the probiotic mixture is safe.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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REFERENCES

Chiu YJ, Nam MK, Tsai YT, Huang CC, Tsai CC (2013). Genotoxicity assessment of multispecies probiotics using reverse mutation, mammalian chromosomal aberration, and rodent micronucleus tests. Sci. World J. http://dx.doi.org/10.1155/2013/254239.

Donohue DC (2006). Safety of probiotics. Asia. Pac. J. Clin. Nutr. 15: 563-569.

Fuller RJ (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66: 365-378.

Hirose Y, Murosaki S, Yamamoto Y, Muroyama K, Miwa Y, Fujishima A, Lynch B. (2009). Safety studies of LP20 powder produced from heat-killed *Lactobacillus plantarum* L-137. Regul. Toxicol. Pharmacol. 54: 214-220.

Jia X, Wang W, Song Y, Li N (2011). A 90-day oral toxicity study on a new strain of *Lactobacillus paracasei* in rats. Food Chem. Toxicol. 49: 1148-1151.

Jia XD, Zhang XP, Li N (2013). A Subchronic Toxicity Study on *Lactobacillus Fermentum* GM 090 in Rat. Biomed. Environ. Sci. 26: 680-683.

Jones ML, Martoni CJ, Tamber S, Parent M, Prakash S (2012). Evaluation of safety and tolerance of microencapsulated *Lactobacillus reuteri* NCIMB 30242 in a yogurt formulation: a randomized, placebo-controlled, double-blind study. Food Chem. Toxicol. 50:2216-2223.

OECD (2001). Repeated dose 28-day oral toxicity study in rodents. OECD guideline for the testing of chemicals. OECD 407. Organization for Economic Cooperation and Development, Paris.

Owen RA, Heywood R (1986). Age-related variations in renal structure and function in Sprague-Dawley rats. Toxicol. Pathol. 14:158-167.

Rao GN (2002). Diet and kidney diseases in rats. Toxicol. Pathol. 30:651-656.

Reeves PG, Rossw KL, Lndlauf J (1993). Development and testing of the AIN-93 purified diets for rodents: results on growth, kidney calcification and bone mineralization in rats and mice. J. Nutr. 123: 1023-1031.

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fondén R, Saxelin M, Collins K, Mogensen G, Birkeland SE, Mattila-Sandholm T (1998). Demonstration of safety of probiotics -- a review. Int. J. Food Microbiol. 44:93-106.

Schoenmakers ACM, Ritskes-Horringa J, Lemmens AG, Beynen AC (1989). Influence of dietary phosphorus restriction on calcium and phosphorus metabolism in rats. Int. J. Vitam. Nutr. Res. 59: 200-206.

Sulemankhil I, Parent M, Jones ML, Feng Z, Labbé A, Prakash S (2012). *In vitro* and *in vivo* characterization and strain safety of *Lactobacillus reuteri* NCIMB 30253 for probiotic applications. Can. J. Microbiol. 58:776-787.

Swendseid ME (1987). Biochemical assessment of protein and amino acid status. In: Livingston, G.E. (Ed.), Proceedings of a National Conference Sponsored by the Food and Nutrition Council of the American Health Foundation, Nutritional Status Assessment of the Individual, Food and Nutrition press, New York. pp. 205-211.

Szabo NJ, Dolan LC, Burdock GA, Shibano T, Sato S, Suzuki H, Uesugi T, Yamahira S, Toba M, Ueno H (2011). Safety evaluation of *Lactobacillus pentosus* strain b240. Food Chem. Toxicol. 49:251-258.

Tsai CC, Chen MH, Liu TH, Chau CG, Chang LT, Tsai CC, Tsen HY. (2004a). Evaluation of the toxicity of *Lactobacillus acidophilus* LAP5 in a 28-Day feeding study in Wistar rats. J. Food Saf. 24:268-280.

Tsai CC, Liu TH, Chen MH, Tsai CC, Tsen HY (2004b). Toxicity evaluation for an *Enterococcus faecium* strain TM39 *in vitro* and *in vivo*. Food Chem. Toxicol. 42:1601-1609.

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Full Length Research Paper

Lead and cadmium residue determination in spices available in Tripoli City markets (Libya)

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In recent years, there has been a growing interest in monitoring heavy metal contamination in food products. Spices can improve the taste of food and can also be a source of many bioactive compounds but can unfortunately, also be contaminated with dangerous materials, potentially heavy metals. This study was conducted to investigate lead (Pb) and cadmium (Cd) contamination in selected spices commonly consumed in Libya including *Capsicum frutescens* (chili pepper), *Piper nigrum* (black pepper), *Curcuma longa* (turmeric) and mixed spices (HRARAT) which consist of a combination of: *Alpinia officinarum, Zingiber officinale* and *Cinnamomum zeylanicum*. Spices were analyzed by atomic absorption spectroscopy after digestion with nitric acid/hydrogen peroxide. The highest levels of lead (Pb) was found in *Curcuma longa* and *Capsicum frutescens* in wholesale markets (1.05 ± 0.01 mg/kg, 0.96 ± 0.06 mg/kg). Cadmium (Cd) levels exceeded FAO/WHO permissible limit. *C. longa* and *P. nigrum* sold in retail markets had a high concentration of Cd (0.36 ± 0.09, 0.35 ± 0.07 mg/kg, respectively) followed by 0.32 ± 0.04 mg/kg for *C. frutescens*. Mixed spices purchased from wholesale markets also had high levels of Cd (0.31 ± 0.08 mg/kg). *C. longa* and *C. frutescens* may pose a food safety risk due to high levels of lead and cadmium. Cadmium levels exceeded FAO/WHO recommendations (0.2 ppm) for *P. nigrum, C. alonga* and HRARAT.

Key words: Heavy metals, lead, cadmium determination, spice, Libya.

INTRODUCTION

In the last decade, interest has grown concerning the dangerous effects of heavy metals on human health resulting from environmental pollution and the prevalence of heavy metals in trace food components such as spices that could result from environmental exposure from the atmosphere, soil and water that eventually find its way into food creating a health risk for humans and animals (Kabata-Pendias, 2011). These toxic metals reach agricultural crops during cultivation, or through industrial activities such as mining, from industrial waste, waste water, pesticides and packaging material (Bradl, 2005; Sarpong et al., 2012; Siegel, 2002). There are several metals of particular concern: lead (Pb), cadmium (Cd), tin (Sn), arsenic (As) and mercury (Hg). Cadmium and lead are

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among the most toxic (Siegel, 2002; Sullivan et al., 2007) due in part to the fact that they accumulate in biological tissues and increase from lower to higher trophic levels, a phenomenon known as biomagnification (Sullivan et al., 2007). Heavy metal damages human health in two ways. The first is disruption of normal cellular processes leading to toxicity. The second, particularly for cadmium and lead is bioaccumulation, particularly in the liver or kidney, where these metals are excreted at a slower rate as compared to uptake (Apostoli, 2006; Zelikoff and Thomas, 1998).

Important food sources of toxic metals are plant foods including spices. Spices are derived from buds, barks, rhizomes, fruits, seeds and other parts of the plant (Peter, 2001). Spices are responsible for making food dishes more distinctive, palatable and aromatic and may contain toxic metals derived from the surroundings through production, processing and marketing (Inam et al., 2013; Ziyaina, 2007). The use of spices and herbs has increased markedly in most regions of the world, including Europe and North America (Nkansah, 2010) with importation from South Asia and developing countries increasing. Several recent food borne incidents have involved both intentionally and unintentionally adulterated spices and herbs with heavy metals added to improve color. Contaminated spices are dumped onto markets in developing countries that have limited ability to test for adulterants. Due to the risk, it is important to evaluate the levels of lead and cadmium in milled spices (red pepper, black pepper, turmeric and mixed spices (HARART) that are commonly consumed in Libya and have a history of adulteration and also to determine the sources and distribution of these metal contaminants in milled species.

Libya, outside of Egypt, is the largest market for spices in North Africa. Central markets in Tripoli serve the entire region from Algeria to Tunisia and into Chad and countries further south and west. India commands 40% of the world chilli market, 11% of the turmeric market and 5% of the black pepper market (www.marketsandmarkets.com). Much of the *Capsicum* spp. and *Piper* spp. sold in Libya is sourced from India, Pakistan and Turkey. High levels of contamination with toxins and filth have been previously reported (fda.gov) possibly due from contaminated irrigation water, and as a result of these perceived risks, a survey was conducted in the Libyan market to assess potential food safety risk.

The objective of this study was to determine the prevalence of lead and cadmium in selected ground spices available in Libyan retail markets.

MATERIAL AND METHODS

Sample collection

Table 1 lists the spices and the part of the plant used. Some of the most consumed spices in Libya include *Capsicum frutescens*, *Piper nigrum, Curcum alonga* and mixed spices (Ziyaina, 2007). The study focused on the contamination assessment of spices that are imported and traded within Libyan markets in 2011. Twenty four samples of each type of spice were collected upon the arrival of these spices to Libya from seven wholesale markets, which are the main sources of spices entering into the country. An additional 36 samples for each type of spice were collected from several retailers in metropolitan Tripoli, Libya.

Sample preparation

Homogenized spice samples were dried in an oven at 100°C for 24 h and then 5 g was accurately weighed into a beaker. Concentrated nitric acid (HNO_3) (65%) was added (5.0 ml) followed by 2.5 ml of 30% hydrogen peroxide (H_2O_2). Samples were left at room temperature for a few minutes and then heated on an electric heater (120°C) and mixed. Five (5) ml of 65% HNO_3 was added and the digests reheated (120°C, few minutes), followed by the addition 10 ml of distilled water. Sample digests were then filtered through Whatman No. 42 filter paper and <0.45 ml and diluted to volume (Yash, 1998). Pb (217 nm) and Cd (228.8 nm) were determined by atomic absorption spectrometry (Varian AAS240, USA). The standard solution for analyses and development of a calibration curve was prepared by diluting a stock solution of 1000 mg L^{-1} of the examined heavy metals. All chemicals used in this experiment were from Sigma Aldrich, St. Louis MO, USA).

Statistical analysis

Means and standard deviations were computed using SAS.LMC. statistical software (SAS Institute Inc, Cary, NC USA) and the Duncan's multiple range test (MRT).

RESULTS AND DISCUSSION

The range and average Pb and Cd in spices from two sources (retailer and wholesale markets) are presented in Tables 2 and 3.

In retail markets, a significant ($P<0.01$) number of spices contained lead. *C. longa* and *C. frutescens* from wholesale markets had the highest levels of contamination. Mixed spices had the lowest concentration of lead, but the levels were still relatively high (0.65 ± 0.09 mg/kg). These values were below the maximum permissible level (10 ppm) recommended by FAO/WHO (2006). Nevertheless, it is important to take the necessary steps to perform routine monitoring of the levels of lead in these spices in order to avert a public health risk since high levels of Pb have been found in other studies on herbs and spice plants from different parts of the world (Seddigi et al., 2013). Chizzola and others (2003) found that heavy metals including Pb were generally within an acceptable range in herbs, spices and medicinal plants on Austrian markets (Chizzola et al., 2003). On the other hand, studies conducted in Poland found the average lead content to be about 1.49 mg/kg in cinnamon, which exceeds the maximum permissible level (Krejpcio et al., 2007).

As shown in Table 2, mixed spices sold in retail markets have a high concentration of Cd (0.36 ± 0.09 mg/kg). The Cd content in black pepper, turmeric and mixed spices were over the maximum permissible limit

Table 1. The names of spices surveyed.

Scientific name	Commercial name	Part used
Capsicum frutescens	Red Pepper	Fruits
Piper nigrum	Black pepper	Seeds
Curcuma longa	Turmeric	Rhizome
Mixed spices		
Alpiniaofficinarum	Galangal	Rhizome
Zingiberofficinale	Ginger	Rhizome
Cinnamomumzeylanicum	Cinnamon	Bark

Table 2. Concentration of lead (Pb) in spices from Libyan markets.

Spice	Element (mg/kg)	Source	Max.	Min.	Average ± S.D
Capsicum frutescens	Pb	Wholesale	0.95	0.81	0.88 ± 0.07
		Retailer	1.02	0.90	0.96 ± 0.06
Piper nigrum	Pb	Wholesale	0.80	0.66	0.73 ± 0.07
		Retailer	0.90	0.69	0.82± 0.13
Curcuma longa	Pb	Wholesale	0.70	0.56	0.63 ± 0.01
		Retailer	1.06	0.96	1.005 ± 0.01
*Mixed spices	Pb	Wholesale	1.00	0.84	0.92 ± 0.08
		Retailer	0.74	1.04	0.89 ± 0.09

*Mixed spices: *Alpinia officinarum, Zingiber officinale* and *Cinnamomum zeylanicum.*

Table 3. Concentration of cadmium (Cd) in spices from Libyan markets.

Spice name	Element (mg/kg)	Source	Maximum limit	Minimum limit	Average ± S.D
Capsicum frutescens	Cd	Wholesale	0.17	0.06	0.14 ± 0.01
		Retailer	0.22	0.16	0.19 ± 0.08
Piper nigrum	Cd	Wholesale	0.19	0.11	0.15 ± 0.07
		Retailer	0.39	0.25	0.32 ± 0.04
Curcuma longa	Cd	Wholesale	0.24	0.14	0.19 ± 0.08
		Retailer	0.39	0.32	0.35 ± 0.07
Mixed spices	Cd	Wholesale	0.40	0.22	0.31 ± 0.08
		Retailer	0.51	0.42	0.36 ± 0.09

(0.2 ppm) recommended by FAO/WHO 2006 (Table 2). The average values of Cd were close to those found in a study by Bempah (2012) that reported the highest concentration of Cd to be 0.44 mg/kg in *Ocimum viride*. The values found here for Libyan markets are high, but lower than what has been found in other markets. In one study in India, the average concentrations of Cd in medicinal plants and spices ranged from 0.684 to 2.751 mg/kg (Subramanian et al., 2012). Other studies have found high Cd concentrations in *Piper nigrum* (206 mg kg^{-1}) and cinnamon *(*124 mg kg^{-1}). Cadmium concentrations in medicinal plants were variable, but often high: in Italy (10-750 mg kg^{-1}), Egypt (50-300 mg kg^{-1}) and Brazil (<0.2-0.74 mg kg^{-}) (Abou-Arab et al., 2000; Caldas et al., 2004).

In general, heavy metal content in spices reflects

environmental pollution levels, bioaccumulation in plant tissue, application of lead or heavy metal containing materials such as arsenate based pesticides (Krejpcio et al., 2007; Nkansah, 2010). High levels of heavy metals could be due to the use of heavy metal-containing fertilezers or from a practice of growing plants with sewage sludge (Ibrahim et al., 2012; Inam et al., 2013).

Conclusion

The levels found in this study for lead in *C. longa, C. frutescens*, and mixed spices (*Alpinia officinarum, Zingiber officinale* and *Cinnamomum zeylanicum*) were below those recommended by FAO. However, levels of cadmium exceeded FAO recommendations for *P. nigrum*, *C. longa*, and mixed spices. Differences observed between Cd and Pb levels for spices sold in retail and wholesale markets indicate that the quality of spices across the value chain in Libya is highly variable and that a number of sources supply the market, some of which are contaminated and some of which are not.

Further studies should be conducted to estimate intake of these and other spices by consumers in the Libya and regionally where a similar cuisine is eaten and where there is a similar lack of control on imported ingredients to ascertain whether there is a health risk.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Abou-Arab AA, Abou Donia MA (2000). Heavy metals in Egyptian spices and medicinal plants and the effect of processing on their levels. J. Agric. Food. Chem. *48*(6):2300-2304.

Apostoli P (2006). Elemental speciation in human health risk assessment. Geneva: Geneva World Health Organization.

Bradl HB (2005). Heavy metals in the environment. Amsterdam: Elsevier Academic Press.

Caldas ED, Machado LL (2004). Cadmium mercury and lead in medicinal herbs in Brazil. Food Chem. Toxicol. *42*(4):599-603.

Chizzola R, Michitsch H, Franz C (2003). Monitoring of metallic micronutrients and heavy metals in herbs, spices and medicinal plants from Austria. Eur. Food Res. Tech. 216(5):407-411.

Ibrahim GI, Hassan LM, Baban SO, Fadhil SS (2012). Effect of Heavy Metal Content of some Common Spices Available in Local Markets in Erbil City on Human Consumption. Raf. J. Sci.23(3):106-114.

Inam F, Deo S, Narkhede N (2013). Analysis of minerals and heavy metals in some spices collected from local market. J.of. Phar.and Bio.Sci. 8(2):40-43.

Kabata-Pendias A (2011). Trace elements in soils and plants. Boca Raton: CRC Press.

Krejpcio Z, Krol E, Sionkowski S, Krejpcio Z, Krol E, Sionkowski S (2007). Evaluation of heavy metals contents in spices and herbs available on the Polish market. Polish J. Environ. Stud. 16(1):97-100.

Nkansah MA (2010). Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. AJSIR American.J.of. Scie. and. Indus. Res. 1(2):158-163.

Peter KV (2001). Handbook of herbs and spices. Cambridge: Woodhead.

Sarpong K, Dartey E, Boateng G, Dapaah H (2012). Profile of hazardous metals in twenty (20) selected medicinal plant samples sold at Kumasi central market, Ashanti region, Ghana.

Seddigi ZS, Kandhro, GA, Shah F, Danish E, Soylak M (2013). Assessment of metal contents in spices and herbs from Saudi Arabia. Toxicol. Ind. Health.

Siegel FR (2002). Environmental geochemistry of potentially toxic metals. Berlin; New York: Springer.

Subramanian R, Gayathri S, Rathnavel C, Raj V (2012). Analysis of mineral and heavy metals in some medicinal plants collected from local market. Asian Pacific. J. Tropical Bio. S74-S78.

Sullivan PJ, James JC, Franklin JA, Paul ER (2007). Toxic legacy synthetic toxins in the food, water, and air of American cities.

Yash K (1998). Handbook of reference methods for plant analysis. Boca Raton: CRC Press.

Zelikoff JT, Thomas PT (1998). Immunotoxicology of environmental and occupational metals. London Bristol, PA: London Bristol, PA: Taylor & Francis.

Ziyaina M (2007). Effect of packaging materials and the change in temperature on microbial load in some spices. Master Thesis Tripoli University Libya 14,28.

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