

Full Paper

The production and shelf life of high-iron, pre-cooked rice porridge with ferrous sulphate and other high-iron materials

Chowladda Teangpook* and Urai Paosangtong

Institute of Food Research and Product Development, Kasetsart University, P.O. Box 1043
Kasetsart, Bangkok 10903, Thailand

* Corresponding author, e-mail: ifrcdt@ku.ac.th

Received: 11 June 2010 / Accepted: 20 August 2011 / Published: 22 August 2011

Abstract: The production and shelf life of high-iron, dried, pre-cooked rice porridge with ferrous sulphate and other high-iron materials was studied. Broken brown rice was soaked in water and ferrous sulphate was added at 0.05, 0.1 and 0.15% of the dried brown rice. The mixture was steamed for 20 min and dried in a double drum dryer. Green shallot, young ginger and cooked chicken fillet were dried in an electric cabinet dryer. Chicken blood and edible fern were dried in a double drum dryer and vacuum freezer respectively. The optimum ferrous sulphate added to the rice was 0.05% and the developed formulation of dried porridge consisted of ferrous sulphate rice (67.80%), chicken fillet (20%), chicken blood (3%), green shallot (0.7%), young ginger (1%), edible fern (0.5%), pepper powder (0.5%), sucrose (3%), salt (3%) and monosodium glutamate (0.5%). The dried porridge had a high iron content of 10.18 mg/50 g and the shelf life was three months at room temperature when stored in either aluminum foil laminated bag or metalite bag.

Keywords: pre-cooked rice porridge, ferrous sulphate, iron fortification

INTRODUCTION

Iron deficiency anemia is still considered the number one nutritional deficiency worldwide [1-2]. A survey by the Department of Health, Thailand revealed that from 1995 to 2003, iron-deficiency anemia had increased by 50% in infants aged 6-11 months, and in a second group consisting of elders, school-age children, preschool-age children and pregnant women, it had increased by over 20%. Iron-fortified foods have been a generally used strategy to combat iron deficiency throughout the world, especially cereal flour (wheat and maize), which is currently the most common vehicle for

iron fortification [3-6]. Condiments and sauces have also been widely consumed as a means of iron fortification where central processing of staple foods is absent. The following examples can be mentioned: curry powder fortified with NaFeEDTA [7]; sugar with NaFeEDTA [8]; salt fortified with ferric orthophosphate, ferrous fumarate, sulphate or bisglycine chelate [9-10]; and soya sauce and fish sauce fortified with NaFeEDTA [11-12].

Porridge is a dish made by boiling oats (rolled, crushed or steel cut) or other grains or legumes in water or milk (or both). It is usually served hot in a bowl or dish. Porridge is a simple and staple dish, especially for breakfast in Thailand. Dried porridge in Thailand is a semi-instant food and a specially controlled product with its quality set according to the Ministry of Public Health [13]. Nowadays, it is a popular food item for every age group, especially children; there are many brands available in Thailand.

Rice is a staple food for half of the world's population including those in Thailand. Thailand's paddy rice output was 31.65 million tonnes in 2008/9 and the country was the world's biggest rice exporter when sales reached 13.09 million tonnes [14]. Rice in Thailand is therefore cheap and suitable for processing. In particular, porridge from brown rice has become popular because it has a high mineral and vitamin content, especially of iron and phytate, which are about 5 and 2.5 times higher respectively than that in white rice [15]. Phytic acid, however, shows a very marked inhibitory effect on the absorption of non-heme Fe in humans [16].

The objective of the current study is to investigate the production, quality and shelf life of high-iron, pre-cooked rice porridge from brown rice fortified with ferrous sulphate and other high-iron materials. Ferrous sulphate containing 32% iron (w/w) has been used in several cases of iron supplementation in such products as parboiled glutinous rice [17], cereal porridge [18], iron supplemented product for schoolchildren [19] and ferrous sulphate tablets used by the Department of Health [20].

MATERIALS AND METHODS

Raw Materials

Supanburi-1 brown rice was sourced from the Department of Agriculture, Thailand. Green shallot, young ginger, chicken fillet, chicken blood, edible fern and other flavouring agents (sugar, salt, soya sauce, white blended pepper powder and monosodium glutamate) were purchased from local markets. Ferrous sulphate powder (food grade) was sourced from Asia Drug & Chemical Ltd. Metalite bags (OPP30u and MCPP25u), 10.8×18.8 cm (water vapour transmission rate of 0.10 g/m²/day), were purchased from a local market. Aluminum foil laminate bags (OPP20/ALU9/LLDPE71), 11×18 cm and 100±10 µm in thickness, were purchased from B.T.T. Thailand Co. Ltd.

Preparation of Dried Ingredients

Fresh green shallot and fresh young ginger were cut into small pieces of about 0.3 cm and 2×0.2×0.2 cm respectively. They were then dried in an electric cabinet dryer at 50°C. Chicken fillets were blended with a blender for about 2 min and 1% salt, 5% soya sauce and 50% water by weight

were there added. The mixture was cooked on a gas stove and dried in an electric cabinet dryer at 60°C. Chicken blood was blended with a blender and dried in a double drum dryer at 140°C and 8 rpm, with a 0.20-mm nip gap. Fresh edible fern was cut into small pieces (about 1 cm), kept at -20°C and dried in a vacuum freeze drier (LyoLab 3000). The dried ingredients were weighed and stored in a refrigerator at 10°C until use. The rate of rehydration (RHD) of the dried ingredients was determined in triplicate by adding 50 parts by weight of hot water (80°C), soaking for 20 min and filtering with a 40-mesh screen for 5 min. The RHD value was taken as weight of rehydrated material/weight of dried material (method modified from Champagne et al. [21]). All dried ingredients were blended in a blender for about 2 min and analysed in triplicate for water activity (a_w) with a water-activity measuring system (Novasina, type a_w -box, Model Ubersicht, No. 861168, Switzerland), and for colour with a spectrophotometer (Spectraflash 600 plus, Data-color International, USA). The CIE colour values recorded were: L^* = lightness (0 = black, 100 = white); a^* (- a^* = greenness, + a^* = redness); and b^* (- b^* = blueness, + b^* = yellowness). The iron content in the chicken fillet and dried chicken blood was determined by AOAC method [22].

Fortification of Rice with Ferrous Sulphate

A completely randomised experimental design with two replications was used. Broken brown rice (200 g) was soaked in water 2.5 times its weight for 3 h and dried ferrous sulphate was added at 0.05, 0.1 and 0.15% (w/w) of the dried brown rice. The samples were steamed for 20 min and placed in a double drum dryer at 140°C and 4 rpm, with 0.20 mm nip gap. The dried iron fortified rice was analysed by the same procedure detailed above. It was then mixed with other dried ingredients to afford the following composition: dried iron fortified rice (67.83%), seasoned chicken fillet (20%), chicken blood (3%), green shallot (0.7%), young ginger (1%), edible fern (0.5%), white blended pepper powder (0.5%), sugar (3%), salt (3%) and monosodium glutamate (0.5%). The mixture was added to 8 parts by weight of hot water (80°C) and boiled for 1 min. A panel of 20 people (24-55 years old) carried out a sensory evaluation which involved appraisal for colour, aroma, flavour, texture and overall preference, using a 7-point hedonic scale (1 = dislike very much, through to 7 = like very much). The data were analysed using analysis of variance and Duncan's new multiple range test for mean comparisons at the 0.05 level of significance, using the SPSS statistical software version 12. The optimum dried iron fortified porridge was analysed for iron, moisture, fat, protein (Kjeldahl method with 6.25 as conversion factor), crude fibre, ash, and carbohydrate according to AOAC methods [22].

Shelf Life of Dried Iron Fortified Porridge

The dried porridge samples produced were packed in aluminum foil laminate (A) bags and metalite (M) bags (30 g/bag) and kept in a darkened drawer at room temperature for 6 months (April–September 2007). Each month, the samples were subjected to tests for colour, a_w , thiobarbituric acid [TBA] [23], microbiological total plate count [24], yeast and mould [25], *Escherichia coli* [26], *Staphylococcus aureus* [27], *Clostridium perfringens* [28], *Bacillus cereus*

[29] and *Salmonella sp* [30], and also to a sensory test of the rehydrated product as detailed above. Analysis of the data for both packaged products followed the process described above.

RESULTS AND DISCUSSION

Preparation of Dried Ingredients

The yields of the dried products were 9.40, 8.80, 9.62, 28.68 and 6.22% of the fresh materials for green shallot, young ginger, edible fern, chicken fillet and chicken blood respectively. Some qualities observed for of the dried ingredients are shown in Figure1. The product colours were: rice - white; young ginger - light yellow; chicken fillet - light brown; green shallot and edible fern - green; and chicken blood - dark brown. The water activity in the samples ranged between 0.22 and 0.40. Since no microorganisms can proliferate if the water activity is less than 0.50 [31], the dried products could be classified as shelf stable. The RHD of edible fern was highest (about 22.10) and that of chicken fillet lowest (4.48) because its texture was lumpy and very hard.

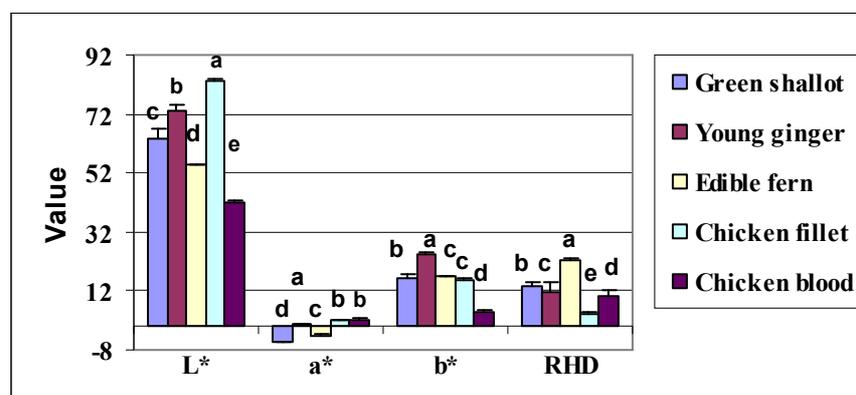


Figure 1. Colour values (L^* , a^* , b^*) and RHD (average \pm standard deviation) of dried ingredients. Means of ingredients with the same letter in each attribute were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

Fortification of Rice with Ferrous Sulphate

The results of the colour analysis of the dried ferrous sulphate fortified rice are shown in Figure 2. Addition of ferrous sulphate, which is brown in colour, caused a reduction in L^* , but increased b^* while a^* (0.39-0.49) was not changed significantly. The results agreed with those of Theuer [18], who found that iron added as ferrous sulphate at 500 mg/kg to rice, oat, whole grain wheat or proanthocyanidin-free barley porridges caused significant changes in the L^* , a^* and b^* values, but the visual appearance of these porridges remained satisfactory.

The results of the sensory evaluation of the rehydrated porridge (mixed with the other ingredients) are shown in Figure 3. The scores were not significantly different except for odour, which was reduced when the level of ferrous sulphate was increased, with 0.05% level registering the highest score. Most attributes had scores from 'like a little' to 'like moderately', especially texture since the porridge was made from brown rice with a high crude fibre content (approximately twice that in polished rice) [15] and so its texture was rather rough. The results of iron analysis of the

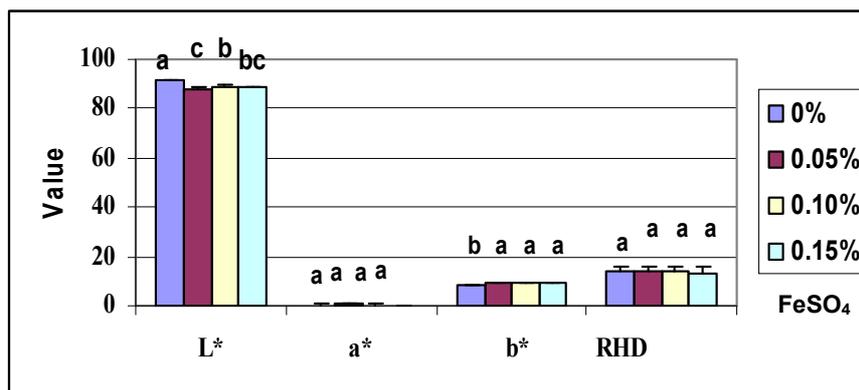


Figure 2. Colour values (L*, a*, b*) and RHD (average \pm standard deviation) of rice with different percentages of ferrous sulphate. Means of each treatment with the same letter were not significantly different ($p > 0.05$). (— indicates the upper range of the mean plus standard deviation.)

dried porridge were 10.18, 20.10 and 30.16 mg/50-g bag for the three treatments of 0.05, 0.1 and 0.15% fortification respectively. The main iron component in the porridge came from the ferrous sulphate, although this was supplemented by other high heme-iron ingredients, namely dried chicken blood and dried chicken fillet with 1,499 and 122 mg/kg respectively. Also, the porridge had non-heme iron from edible fern (36.3 mg/kg fresh weight) and brown rice itself (7.71 mg/kg) [32]. Non-heme iron, however, is poorly absorbed compared with heme iron [33].

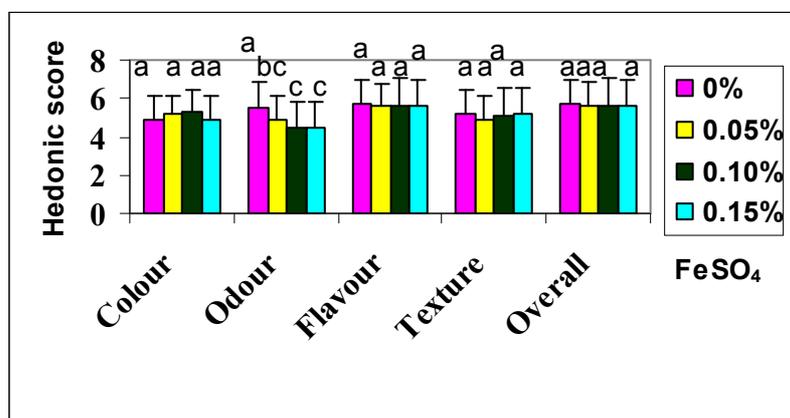


Figure 3. Sensory evaluation (average \pm standard deviation) of the quality of rehydrated porridge. Means of each treatment for each attribute with different letters were significantly different at $p \leq 0.05$.

In general, the Thai people should receive iron at a rate of 10-15 mg/day [34]. Thus, the amount of iron (10.18 mg/50-g dried porridge) supplied by the 0.05% fortification treatment should be sufficient since people may receive additional iron from other foods consumed in other meals during the day. Among the elderly, intake of highly bioavailable forms of iron (supplementary iron and red meat), fruits and non-heme iron absorption enhancer (vitamin C) can promote high iron stores. The risk of high iron stores is significantly higher in subjects who take in more than 30 mg of

supplementary iron per day than in nonusers, whereas foods containing phytate (whole grains) decrease these stores [35]. High iron stores may be associated with many chronic diseases such as heart disease [36–37] and cancer [38]. The demand for iron, however, differs according to sex and age, e.g. 8.1 mg/day for 6-8 year-olds, 11.8 mg/day for 9-12 year-olds and 28.2 mg/day for girls 13-15 years old [34]. Thus, having the highest score of sensory evaluation for odour and optimum amount of iron, the 0.05% ferrous sulphate fortification treatment was selected for the study of shelf life. The correlations between colour, odour, flavour, texture and overall impression are shown in Table 1 with all attributes correlated, especially texture and overall impression.

Table 1. Pearson correlation coefficients of sensory evaluation at probability levels of $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

	Colour	Odour	Flavour	Texture	Overall
Colour	1				
Odour	0.380**	1			
Flavour	0.383**	0.581**	1		
Texture	0.389**	0.442**	0.556**	1	
Overall	0.375**	0.463**	0.692**	0.745**	1

The chemical composition (% dry weight) of the optimally iron fortified dried porridge was 6.34 ± 1.10 , 65.85 ± 2.56 , 19.16 ± 0.88 , 2.30 ± 0.81 , 5.10 ± 0.33 and 6.35 ± 0.2 % for moisture, carbohydrate (including crude fibre), protein (factor 6.25), fat, dietary fibre and ash respectively. The moisture content of the product was lower than 10% while also having a high protein content of more than 8%, the minimum value set out by the Ministry of Public Health [13]. The main protein source of the porridge was chicken fillet, which was also effective at enhancing iron absorption [39]. The product therefore could be claimed to be an iron source as well as a protein source.

Shelf Life of Iron Fortified Porridge

Based on the analysis during the months of storage, the only feature of the dried porridge in the two packaging systems that was significantly different was the water activity,. The product's water activity in the lower-quality M-type packages registered higher values than those from the A-type packages. However, all samples had a water activity of less than 0.51, although the value increased with storage time (Figure 4).

The colours of the dried porridge from the two types of packaging were not significantly different, with L^* ranging from 79.80 to 81.23, a^* from 0.11 to 0.33 and b^* from 8.46 to 10.05 (Figures 5-7).

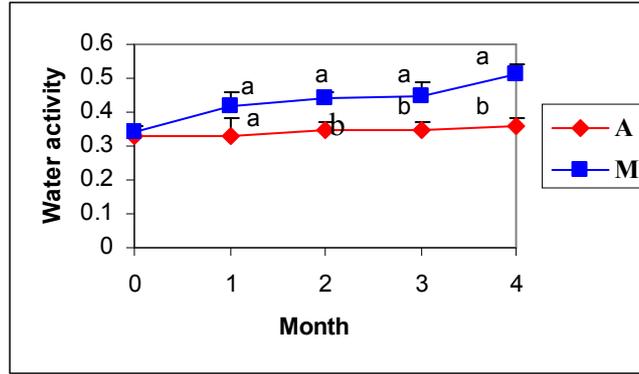


Figure 4. Water activity (average \pm standard deviation) of dried porridge in two types of packaging (A and M bags) plotted against storage month. Means for each type of packaging for each month of storage with the same letter were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

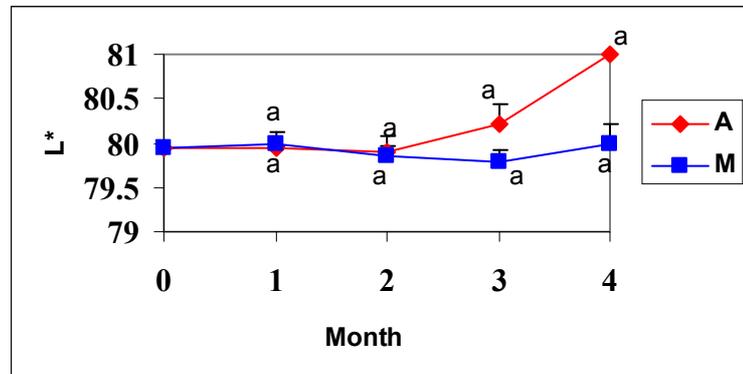


Figure 5. Colour (L^*) values (average \pm standard deviation) of dried porridge in two types of packaging (A and M bags) plotted against storage month. Means for each type of packaging for each month with the same letter were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

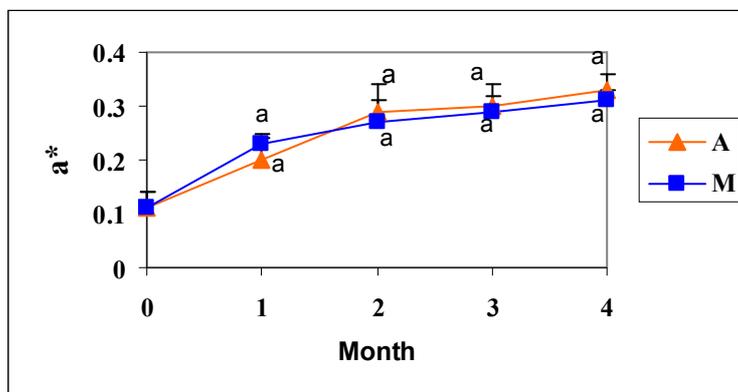


Figure 6. Colour (a^*) values (average \pm standard deviation) of dried porridge in two types of packaging (A and M bags) plotted against storage month. Means for each type of packaging for each month with the same letter were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

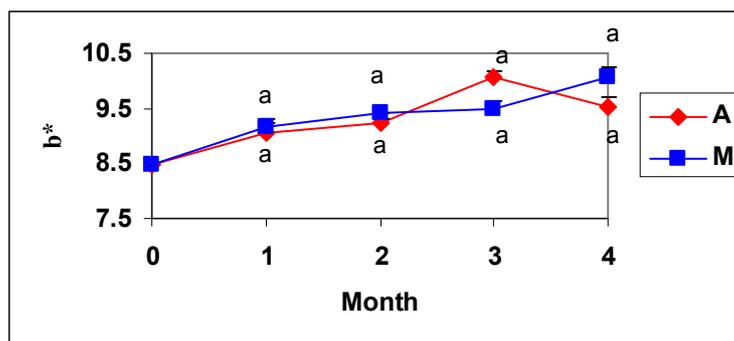


Figure 7. Colour (b^*) values (average \pm standard deviation) of dried porridge in two types of packaging (A and M bags) plotted against storage month. Means for each type of packaging for each month with the same letter were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

Figure 8 shows results of TBA test used for the measurement of fat oxidation [40] in the porridge samples contained in the two types of packaging. Malonaldehyde, an end-product in the autoxidation process, was observed to increase with storage time, with samples in the A-type packages having lower values than those obtained from the M-type packages, although the levels were not significantly different.

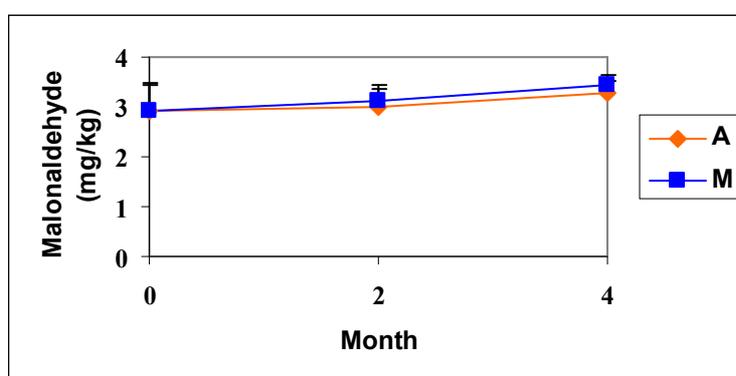


Figure 8. Results of TBA test: malonaldehyde (average \pm standard deviation) in of dried porridge from two types of packaging (A and M bags) plotted against storage month. (\top indicates the upper range of the mean plus standard deviation.)

Results of the monthly sensory evaluation of the rehydrated porridge from the A- and M-type packagings by the panelists are shown in Figure 9. Most scores were not significantly different except those for colour and flavour, especially for the porridge from M-type packaging at the fourth month of storage, which were less than those from A-type packaging. The porridge from the lower-quality, translucent M-type packaging showed some rancidity. Furthermore, in this month, the overall score for the two packaging types reduced markedly, with scores of only 'dislike a little', which suggested that the product could not be kept for more than three months. This is also apparent from the TBA levels (Figure 8) after three months at about 3.3 and 3.1 mg/kg for samples in the M- and A-type packagings respectively. As for the microorganism levels, even though they were still

within the safe ranges in both types of package, there was a clear trend of increasing microbial activity with time (Table 2).

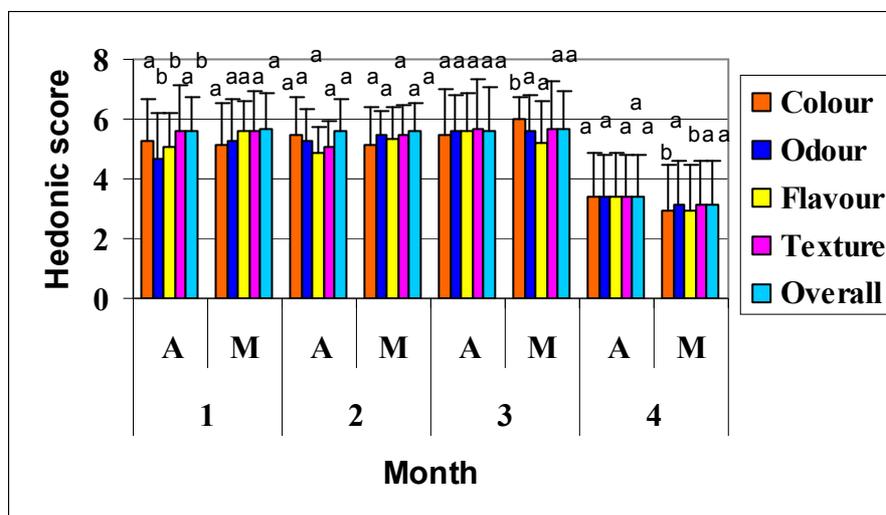


Figure 9. Sensory evaluation (average \pm standard deviation) of the quality of rehydrated porridge from two types of packaging (A and M bags). Means for each type of packaging for each month with the same letters were not significantly different ($p > 0.05$). (\top indicates the upper range of the mean plus standard deviation.)

Table 2. Microbial sampling of dried porridge samples from the shelf life study

Month	Sample	Microorganism	Result
0	A and M	Total plate count (cfu/g)	6.7×10^4
		Yeast and mould (cfu/g)	< 10
		<i>Escherichia coli</i> (MPN/g)	< 3
		<i>Staphylococcus aureus</i> (cfu/g)	< 10
		<i>Clostridium perfringens</i> /0.1 g	Not detected
		<i>Salmonella sp.</i> /25 g	Not detected
		<i>Bacillus cereus</i> (cfu/g)	10
1	A	Total plate count (cfu/g)	7.7×10^4
		Yeast and mould (cfu/g)	< 10
	M	Total plate count (cfu/g)	1.0×10^4
		Yeast (cfu/g)	< 10
2	A	Mould (cfu/g)	20
		Total plate count (cfu/g)	3.2×10^4
		Yeast and mould (cfu/g)	< 10
	M	Total plate count (cfu/g)	1.8×10^5
		Yeast (cfu/g)	< 10
		Mould (cfu/g)	20

Table 2. (Continued)

Month	Sample	Microorganism	Result
3	A	Total plate count (cfu/g)	1.5×10^5
		Yeast (cfu/g)	< 10
		Mould (cfu/g)	30
	M	Total plate count (cfu/g)	5.9×10^4
		Yeast (cfu/g)	< 10
		Mould (cfu/g)	20
4	A	Total plate count (cfu/g)	8.1×10^4
		Yeast (cfu/g)	< 10
		Mould (cfu/g)	20
	M	Total plate count (cfu/g)	8.4×10^4
		Yeast (cfu/g)	< 10
		Mould (cfu/g)	40

CONCLUSIONS

Fortification of rice with ferrous sulphate was found to be optimum at 0.05% of dried brown rice. The dried, seasoned porridge had a high iron content (10.18 mg/50 g) supplied by the ferrous sulphate as well as other high-iron materials (chicken blood, chicken fillet and edible fern), meeting the requirements for a high-iron food product. The shelf life of this product was three months at room temperature in an aluminum foil laminate bag or a metalite bag.

ACKNOWLEDGEMENT

Financial support for this research from the Kasetsart University Research and Development Institute (KURDI) is acknowledged.

REFERENCES

1. S. Denic and M. M. Agarwal, "Nutritional iron deficiency: An evolutionary perspective", *Nutr.*, **2007**, *23*, 603-614.
2. R. J. Stoltzfus, "Defining iron-deficiency anemia in public health terms: A time for reflection", *J. Nutr.*, **2001**, *131*, 565S-567S.
3. UNICEF, "Preventing Iron Deficiency in Women and Children: Technical Consensus on Key Issues", UNICEF/UNU/WHO/MI Technical Workshop, **1998**, New York, USA, pp.29-32.
4. R. Hurrell, T. Bothwell, J. D. Cook, O. Dary, L. Davidsson, S. Fairweather-Tait, L. Hallberg, S. Lynch, J. Rosado, T. Walter and P. Whittaker, "The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Sharing United States Technology to aid in the improvement of nutrition", *Nutr. Rev.*, **2002**, *60*, 391-406.

5. R. F. Hurrell, D. E. Furniss, J. Burri, P. Whittaker, S. R. Lynch and J. D. Cook, "Iron fortification of infant cereals: A proposal for the use of ferrous fumarate or ferrous succinate", *Am. J. Clin. Nutr.*, **1989**, 49, 1274-1282.
6. M. B. Zimmermann, P. Winichagoon, S. Gowachirapant, S. Y. Hess, M. Harrington, V. Chavasit, S. R. Lynch and R. F. Hurrell, "Comparison of the efficacy of wheat-based snacks fortified with ferrous sulphate, electrolytic iron, or hydrogen-reduced elemental iron: Randomized, double-blind, controlled trial in Thai women", *Am. J. Clin. Nutr.*, **2005**, 82, 1276-1282.
7. D. E. Ballot, A. P. MacPhail, T. H. Bothwell, M. Gillooly and F. G. Mayet, "Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: Report of a controlled iron-fortification trial", *Am. J. Clin. Nutr.*, **1989**, 49, 162-169.
8. F. E. Viteri, E. Alvarez, R. Batres, B. Torun, O. Pineda, L. A. Mejía and J. Sylui, "Fortification of sugar with iron sodium ethylenediaminetetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations", *Am. J. Clin. Nutr.*, **1995**, 61, 1153-1163.
9. Working Group on Fortification of Salt with Iron, "Use of common salt fortified with iron in the control and prevention of anemia: A collaborative study", *Am. J. Clin. Nutr.*, **1982**, 35, 1442-1451.
10. H. Foy, "Fortification of salt with iron", *Am. J. Clin. Nutr.*, **1976**, 29, 935-936.
11. X. Yang, Y. Tian, J. Huo and J. Piao, "Iron absorption of NaFeEDTA in soy sauce in Chinese female", Proceedings of Conference on Forging Effective Strategies to Combat Iron Deficiency, **2001**, Atlanta, USA, Abstract M8.
12. P. V. Thuy, J. Berger, L. Davidsson, N. C. Khan, T. T. Nga, N. T. Lam, T. T. Mai, C. Flowers, Y. Nakanishi, J. D. Cook, R. F. Hurrell and H. H. Khoi, "Regular consumption of NaFeEDTA fortified fish sauce improves iron status of anemic Vietnamese women", Proceedings of Conference on Forging Effective Strategies to Combat Iron Deficiency, **2001**, Atlanta, USA, Abstract M10.
13. Ministry of Public Health, "Announcement No 210", <http://www.fda.moph.go.th/fda-net/html/product/food/ntfmoph/ntf210.htm>, **2000** (Accessed: May 23, 2010).
14. C. Damrongkiat, "Thai rice research, crisis and opportunity", <http://www.thairice.org/news/TRFnews/doc/TRresearch1.ppt#366,6>, **2010** (Accessed: May 23, 2010).
15. N. Boontaveeyuwat and D. Keawsiri, "Does brown rice decrease iron availability of rice based meals?", *J. Nutr. Assoc. Thailand*, **2004**, 39, 10-16.
16. A.-S. Sandberg and U. Svanberg, "Phytate hydrolysis by phytase in cereals; Effects on in vitro estimation of iron availability", *J. Food Sci.*, **1991**, 56, 1330-1333.
17. P. Wiriya-Charree, C. Techapun, S. Pruenglampoo, W. S. Wattanutchariya, S. Jongkaewwattana and R. Techapun, "Increasing nutritive value of parboiled glutinous rice", *J. Nation. Res. Council. Thailand (Soc.)*, **2007**, 39, 65-75 (in Thai).
18. R. Theuer, "Effect of iron on the color of barley and other cereal porridges", *J. Food Sci.*, **2002**, 67, 1208-1211.
19. R. Sungthong, L. Mo-Suwan, V. Chongsuvivatwong and A. F. Geater, "Once weekly is superior to daily iron supplementation on height gain but not on hematological improvement among schoolchildren in Thailand", *J. Nutr.*, **2002**, 132, 418-422.

20. Bureau of Drug Control, "Ferrous sulphate, nourishing blood tablets", http://www2.fda.moph.go.th/drug/zone_drug/dru001.asp?title=3&display=a16b1603 (Accessed: May 23, 2010).
21. D. A. Smith, R. M. Rao, J. A. Liuzzo and E. Champagne, "Chemical treatment and process modification for producing improved quick-cooking rice", *J. Food Sci.*, **1985**, 50, 926-931.
22. W. Horwitz (Ed.), "Official Methods of Analysis", 17th Edn., Association of Official Analytical Chemists, Gaithersburg, **2000**.
23. B. G. Tarladgis, B. M. Watts and M. Yonathan, "Distillation method for the determination of malonaldehyde in rancid foods", *J. Am. Oil Chem. Soc.*, **1960**, 37, 44-48.
24. L. Maturin and J. T. Peeler, "Bacteriological analytical manual: Aerobic plate count", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063346.htm>, **2001** (Accessed: June 20, 2010).
25. V. Tournas, M. E. Stack, P. B. Mislivec, H. A. Koch and R. Bandler, "Bacteriological analytical manual: Yeasts, molds and mycotoxins", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071435.htm>, **2001**, (Accessed: June 20, 2010).
26. P. Feng, S. D. Weagant and M. A. Grant, "Bacteriological analytical manual: Enumeration of *Escherichia coli* and the coliform bacteria", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm>, **2002** (Accessed: June 20, 2010).
27. R. W. Bennett and G. A. Lancette, "Bacteriological analytical manual: *Staphylococcus aureus*", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071429.htm>, **2001** (Accessed: June 20, 2010).
28. E. J. Rhodehamel and S. M. Harmon, "Bacteriological analytical manual: *Clostridium perfringens*", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070878.htm>, **2001** (Accessed: June 20, 2010).
29. E. J. Rhodehamel and S. M. Harmon, "Bacteriological analytical manual: *Bacillus cereus*", <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070875.htm>, **2001** (Accessed: June 20, 2010).
30. "ISO 6579:2002 - Microbiology of food and animal feeding stuffs: Horizontal method for the detection of *Salmonella spp.*", 4th Edn., International Organization for Standardization, Switzerland, **2002**.
31. L. R. Beuchat, "Microbial stability as affected by water activity", *Cereal Foods World.*, **1981**, 26, 345-349.
32. Department of Health, "Nutritive Values of Thai Foods", Nutrition Division, Ministry of Public Health, Bangkok, **2001** (in Thai).
33. L. Hallberg and L. Hulthen, "Prediction of dietary iron absorption: An algorithm for calculating absorption and bioavailability of dietary iron", *Am. J. Clin. Nutr.*, **2000**, 71, 1147-1160.
34. The Committee on Recommended Daily Dietary Allowances, "Dietary Reference Intake for Thais 2003", Ministry of Public Health, Bangkok, **2003**, p.345.

35. D. J. Fleming, K. L. Tucker, P. F. Jacques, G. E. Dallal, P. W. Wilson and R. J. Wood, “Dietary factors associated with the risk of high iron stores in the elderly Framingham Heart Study cohort”, *Am. J. Clin. Nutr.*, **2002**, 76, 1375-1384.
36. J. T. Salonen, K. Nyyssonen, H. Korpela, J. Tuomilehto, R. Seppanen and R. Salonen, “High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men”, *Circulation*, **1992**, 86, 803–811.
37. T. Tuomainen, K. Punnonen, K. Nyyssonen and J. T. Salonen, “Association between body iron stores and the risk of acute myocardial infarction in men”, *Circulation*, **1998**, 97, 1461-1466.
38. R. G. Stevens, D. Y. Jones, M. S. Micozzi and P. R. Taylor, “Body iron stores and the risk of cancer”, *N. Engl. J. Med.*, **1988**, 319, 1047–1052.
39. M. B. Reddy, R. F. Hurrell, M. A. Juillerat and J. D. Cook, “The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans”, *Am. J. Clin. Nutr.*, **1996**, 63, 203-207.
40. C. G. Sidwell, H. Salwin, M. Benca and J. H. Mitchell Jr., “The use of thiobarbituric acid as a measure of fat oxidation”, *J. Am. Oil Chem. Soc.*, **1954**, 31, 603-606.