## Physicochemical Properties of Protein Hydrolysate from Rice Malt

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#### Abstract

This research was conducted to determine the physical, chemical qualities and functional properties of protein hydrolysate from rice malt, the product from industrial-scale production by spray drying. Rice malt protein hydrolysate showed % protein at 77.5 and mainly consisted of phenolic compound and high level of vitamin B1, B3, B5 and B6. The amino acid composition of rice malt protein hydrolysate was superior to soybean protein isolation, such as alanine, cystein, valine, methionine, leucine and phenylalanine. Moreover, the levels of all essential amino acids met the standard for the child and adult intake recommendation of FAO/WHO. Rice malt protein hydrolysate had excellent solubility at 92.71 %, with low characteristics of foaming property and emulsification. The data from shelf life evaluation of rice malt protein hydrolysate at ambient temperature, 25 °C and 4 °C for 5 years showed that the functional properties and physical qualities except for color value had not changed. However, the antioxidant activities had slightly decreased as the storage time increase especially when stored at ambient temperature.

Keywords: rice protein, protein hydrolysate, rice malt protein hydrolysate, rice malt, functional properties, amino acid composition

## บทคัดย่อ

งานวิจัขนี้ตรวจสอบคุณภาพทางกายภาพ เคมีและสมบัติเชิงหน้าที่ของโปรดีนไฮโดรไลเซทจากข้าวมอลต์ที่ได้จากการผลิตระดับ อุตสาหกรรมโดยผ่านการทำแห้งแบบพ่นฝอย โปรดีนไฮโดรไลเซทจากข้าวมอลด์มีปริมาณโปรดีนเท่ากับร้อยละ 77.5 มีสารฟีนอลิกส์และวิตามินบี1 บี3 บี5 และบี6 ในระดับสูง พบปริมาณกรคอะมิโนองก์ประกอบชนิดอาลานีน ซีสเทอีน แวลีน เมทไซโอนีน ลิวซีนและฟีนิลลาลานีนสูงกว่าโปรตีน ไฮโดรไลเซทจากถั่วเหลือง ยิ่งไปกว่านั้น พบว่ามีปริมาณกรดแอมิโนจำเป็นทุกชนิดอยู่ในระดับมาตรฐานความต้องการของเด็กและผู้ใหญ่ที่แนะนำโดย FAO/WHO มีความสามารถในการละลายน้ำได้ดีเท่ากับร้อยละ 92.71 แสดงความสามารถในการเกิดฟองและดัชนีกิจกรรมการเกิดอิมัลชันในระดับต่ำ ผลการทดสอบความลงตัวของโปรดีนไฮโดรไลเซตระหว่างการเก็บรักษาเปรียบเทียบระหว่างอุณหภูมิสิ่งแวดล้อม 25 และ 4 องศาเซลเซียส เป็นเวลา 5 ปี ไม่พบการเปลี่ยนแปลงสมบัติเชิงหน้าที่และคุณภาพทางกายภาพทุกรายการยกเว้นก่าสี ในขณะที่พบการลดลงของกิจกรรมการเป็นสารด้าน ออกซิเดชันเมื่อระยะเวลาเก็บรักษานานขึ้นโดยเฉพาะเมื่อเก็บที่อุณหภูมิสิ่งแวดล้อม

<mark>ี คำสำคัญ:</mark> โปรตีนข้าว โปรตีนไฮโครไถเซท โปรตีนไฮโครไถเซทข้าวมอลต์ ข้าวมอลต์ สมบัติเชิงหน้าที่ กรคแอมิโนองค์ประกอบ

#### 1. Introduction

Rice is one of the largest food ingredients and represents a staple food for more than half of the world's population. Rice proteins are hypoallergenic, nutritious and healthy for human consumption. Rice proteins top the list in essential amino acids make-up compared to any of the plant proteins. Rice proteins contains the highest level of antioxidative peptides derived from rice bran protein hydrolysates (Adebiyi et al., 2009). Rice protein hydrolysate has special application in sports medicine because its consumption allows amino acids to be absorbed by the body more rapidly than intact proteins do, thus maximizing nutrientdelivery to muscle tissues (Manninen, 2004). It is also used in the biotechnology industry as a supplement to cell cultures (Ummadi & Curic-Bawden, 2010). Up to now, protein hydrolysate from rice has mainly been produced from rice bran by chemical or enzymatic method (Fabian et al., 2010). However, protein hydrolysates made by acid and alkaline hydrolysis of proteins are also commercially available (Pasupuleti & Demain, 2010). Protein hydrolysates from broken rice malt, which are the by-products of rice malt production, have not been reported. The raw material, rice seeds that have gone through germinating can increase many bioactive components such as free amino acid, GABA, phenolic compounds, minerals and vitamin B such as B1, B2, B3, B5, B6 and B9 in a higher quantity compared to un-germinated seeds which were easily extracted and soluble in water. Building on previous research on commercial production

of rice malt extract from Thai rice by temperature adjustment extraction together with commercial enzyme at the industrial scale of batch 15000 liters (Yupakanit et al., 2012),rice malt protein hydrolysate was prepared by inner enzyme of broken rice combination with commercial enzyme. The process was done by mashing method to produce wort for beer production following the method of Yupakanit et al. (2012). Production of value-added product from broken rice malt is to create the new product of "protein hydrolysate", as well as good functional properties, which can pave the way for full utilization.

## 2. Objectives

The objectives of this research were to study the physicochemical properties and functional properties including antioxidant activity and to study shelf life of protein hydrolysate.

### 3. Materials and methods

Raw material : The preparation of the spray drying of rice malt protein hydrolysate powder was provided by Tipco Biotech factory in 2012. Soybean protein hydrolysate and rice protein (Oryza 70) were purchased from Oryza Oil &Fat Chemical Co. Ltd. All chemicals used were of analytical grade.

Quality analysis : In order to determine chemical analysis, antioxidant activity was determined by DPPH radical scavenging method (Daou & Zhang, 2011) . Protein content was established with the use of Kjeldahl method based on AOAC (2000). For nitrogen to protein conversion, the factor of 6.25 was used. In addition, Kjeldahl method was also applied to verify soluble nitrogen concentration in the hydrolysates obtained. Phenolic content was determined by the method of Folin-Ciocalteau phenol test (Singleton & Rossi, 1965). Vitamin B1, B3, B5 and B6 were determined by using HPLC at TISTR. Amino acid composition was determined by using Amino acid analyzer Biochrom 20 (Auto sample version) at TISTR. Electrophoresis was performed in a 6% polyacrylamide gel in a horizontal apparatus (E.C.Co.) with 0.017 M aluminum lactate buffer at pH 3.2. Gel was stained with Amido Black 10B.2 hours at 11 v./cm. For characterization of particle morphology, the shape and surface morphology of the particles were examined by scanning electron microscope (SEM) (JEOL, model JSM-5410LV, Tokyo, Japan) The acceleration voltage was 15 kV and 150X, and 2,000X magnification was used for the samples. In order to determine physical properties, the color was measured by using MinoltaCR10 to obtain the color values (L\*,a\* and b\* values). L\* represents lightness, a\* represents greenness (-) to redness (+), b\* represents blueness (-) to yellowness (+) values. Moisture content was determined according to AOAC (2000). Water activity was measured by AQUA Lab apparatus. In order to determine functional properties, protein solubility, foaming ability and emulsion activity index were calculated by the formula according to the method of Benjakul and Morrissey (1997).

## 4. Results and discussions

It was showed that the content of free amino nitrogen was 12.42% due to the fact that protein stored in germinated seed were decomposed, changed into transportable amino acid molecules, and supplied to the growing embryo of the rice seedling. Protein hydrolysate from rice malt contained free amino nitrogen at 12.42 (%w/w) in the same level of commercial protein hydrolysate solution which had specification not less than 10%. Protein hydrolysate from rice malt contained phenolic compound at 143.15 mg/100g. Tian et al. (2004, 2005) described that large molecule soluble and insoluble phenolic compounds were changed into free soluble small molecules of ferulic acid, sinapinic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, protocatechuic acid, hydroxybenzoic acid, vanillic acid, and syringic acid in rice. The total phenolic content increased nearly 10 times. In addition, vitamin B1, B3, B5 and B6 were found in the high level contents. The result was showed in Table 1.

Table 1 Physical and chemical question	ality of protein hydrolysate	es from rice malt.
Items		contents
Free Amino Nitrogen (%w/w)		12.42± 0.01
Protein content (%)		$77.50 \pm 5.45$
Phenolic compound (mg/100g)		$143.15 \pm 0.00$
Vitamin		<b>A</b>
	B1 (mg/100g)	0.34 ± 0.00 (23% Thai RDI)
	B3 (mg/1000g)	7.44 ± 0.06 (37% Thai RDI)
	B5 (mg/100g)	6.00 ± 0.05 (100% Thai RDI)
	B6 (mg/100g)	0.52 ± 0.00 (26% Thai RDI)

Means of triplicates  $\pm$  standard deviation of mean.

The composition of the amino acids in protein hydrolysates from rice malt is related to the soybean protein hydrolysates (Table 2). Even though the contents of lysine were lower than those of soybean protein hydrolysates, the levels of all essential amino acids met the standard for the child and adult intake recommendation of FAO/WHO/UNU. The levels of alanine, cystein, valine, methionine, leucine and phenylalanine in protein hydrolysates from rice malt were higher than those of soybean protein hydrolysates, valine, methionine, leucine and phenylalanine are necessary for the new-born babies (Wang et al., 2015). Furthermore, the levels of all amino acids except tyrosine and histidine from rice malt were higher than those of commercial rice protein (Oryza70). Therefore, the results of protein hydrolysates from rice malt might be considered as a good resource of essential amino acids for adults or children.

Table 2 Amino	acid compositi	on analysis of	protein hy	drolysates (	(g/100g).

Protein hydrolysates	Protein hydrolysates	Commercial Rice Protein	*FAO/WHO/UNU		
from rice malt	from soybean				
		(Oryza70)	Child	Adult	
10.76 ± 0.13	$11.96 \pm 0.17$	6.92			
$3.53 \pm 0.35$	3.71 ± 0.11	2.88	3.4	0.9	
$4.07\pm0.21$	$5.51 \pm 0.10$	3.92			
$14.85 \pm 0.13$	$20.53\pm0.07$	14.00			
$3.71 \pm 0.37$	$5.28\pm~0.06$	3.70			
3.51 ± 0.12	$4.64\pm~0.09$	3.46			
$4.87\pm~0.08$	3.90 ± 0.11	4.37			
$1.98\pm~0.09$	$1.01 \pm 0.08$	1.92			
$5.14\pm~0.78$	$4.80\pm~0.09$	4.62	3.5	1.3	
$2.82\pm~0.03$	$1.10 \pm 0.07$	2.35	2.5	1.7	
$4.16 \pm 0.03$	$4.93 \pm 0.08$	3.26	2.8	1.3	
	from rice malt $10.76 \pm 0.13$ $3.53 \pm 0.35$ $4.07 \pm 0.21$ $14.85 \pm 0.13$ $3.71 \pm 0.37$ $3.51 \pm 0.12$ $4.87 \pm 0.08$ $1.98 \pm 0.09$ $5.14 \pm 0.78$ $2.82 \pm 0.03$	from rice maltfrom soybean $10.76 \pm 0.13$ $11.96 \pm 0.17$ $3.53 \pm 0.35$ $3.71 \pm 0.11$ $4.07 \pm 0.21$ $5.51 \pm 0.10$ $14.85 \pm 0.13$ $20.53 \pm 0.07$ $3.71 \pm 0.37$ $5.28 \pm 0.06$ $3.51 \pm 0.12$ $4.64 \pm 0.09$ $4.87 \pm 0.08$ $3.90 \pm 0.11$ $1.98 \pm 0.09$ $1.01 \pm 0.08$ $5.14 \pm 0.78$ $4.80 \pm 0.09$ $2.82 \pm 0.03$ $1.10 \pm 0.07$	from rice maltfrom soybean(Oryza70) $10.76 \pm 0.13$ $11.96 \pm 0.17$ $6.92$ $3.53 \pm 0.35$ $3.71 \pm 0.11$ $2.88$ $4.07 \pm 0.21$ $5.51 \pm 0.10$ $3.92$ $14.85 \pm 0.13$ $20.53 \pm 0.07$ $14.00$ $3.71 \pm 0.37$ $5.28 \pm 0.06$ $3.70$ $3.51 \pm 0.12$ $4.64 \pm 0.09$ $3.46$ $4.87 \pm 0.08$ $3.90 \pm 0.11$ $4.37$ $1.98 \pm 0.09$ $1.01 \pm 0.08$ $1.92$ $5.14 \pm 0.78$ $4.80 \pm 0.09$ $4.62$ $2.82 \pm 0.03$ $1.10 \pm 0.07$ $2.35$	from rice maltfrom soybean(Oryza70)Child $10.76 \pm 0.13$ $11.96 \pm 0.17$ $6.92$ $3.53 \pm 0.35$ $3.71 \pm 0.11$ $2.88$ $3.4$ $4.07 \pm 0.21$ $5.51 \pm 0.10$ $3.92$ $14.85 \pm 0.13$ $20.53 \pm 0.07$ $14.00$ $3.71 \pm 0.37$ $5.28 \pm 0.06$ $3.70$ $3.51 \pm 0.12$ $4.64 \pm 0.09$ $3.46$ $4.87 \pm 0.08$ $3.90 \pm 0.11$ $4.37$ $1.98 \pm 0.09$ $1.01 \pm 0.08$ $1.92$ $5.14 \pm 0.78$ $4.80 \pm 0.09$ $4.62$ $3.5$ $2.82 \pm 0.03$ $1.10 \pm 0.07$ $2.35$ $2.5$	

Amino acid	Protein hydrolysates from rice malt	Protein hydrolysates from soybean	Commercial Rice Protein	*FAO/WHO/UNU	
		-	(Oryza70)	Child	Adult
Leucine	$7.04 \pm 0.58$	$1.72 \pm 0.09$	6.54	6.6	1.9
Tyrosine	$3.40\pm\ 0.02$	$3.70\pm~0.08$	3.91		4
Phenylalanine	8.07 ± 0.21	5.41 ± 0.09	4.25	6.3	1.9
Histidine	$1.90\pm\ 0.04$	$2.54\pm0.06$	2.05	1.9	1.6
Lysine	$4.17\pm\ 0.05$	$6.09\pm~0.08$	2.26	5.8	1.6
Arginine	$6.59\pm~0.27$	7.81 ± 0.10	6.29		

\*Essential amino acid requirement for child and adults according to FAO/WHO/UNU (1985)

The functional properties of protein hydrolysates from rice malt was showed in Table 3. Protein hydrolysate from rice malt had excellent solubility at 92.71 % at the same level of commercial protein hydrolysate solution of which specification is completely soluble in water (Priya Chemicals, 2007). Yaowapa et al. (2007) claimed that the percentage of solubility was found to depend on amount of solubilised free amino acids. Therefore, increasing of free amino acids affects increasing of solubility. Foaming property of protein hydrolysate from rice malt was 45.35%. Damodaran (1997) reported that the foaming ability was low at pH 4-6 corresponding with the isoelectric point of protein. For emulsification ability, the EAI value was 10.50 m<sup>2</sup>/g due to pH, which was similar to the report by Wang et al. (2015). They described that the lower EAI values were found at pH 4-6 (isoelectric point) The process was the same condition for rice malt protein hydrolysis of industrial scale in this report in which protein was hydrolyzed at pH 4-5 (Yupakanit et al., 2012). The emulsification ability is positively correlated with the electrostatic repulsion and net charge, in which they influence the distribution of oil/water interface, the interfacial energy of the emulsion droplet, and the stability of the surface hydrophobicity and conformation.

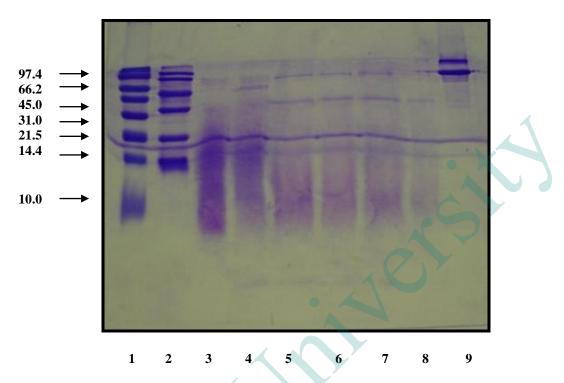
Table 3 Functional properties of protein hydrolysates from rice malt

Items	Contents
Solubility (%)	$92.71 \pm 0.47$
Foaming ability (%)	45.35± 1.12
Emulsion activity index $(m^2/g)$	10.50± 0.85

Means of triplicates  $\pm$  standard deviation of mean

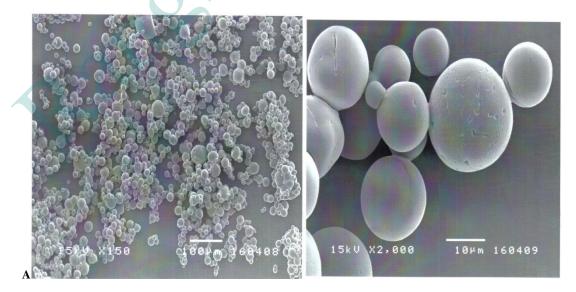
SDS-PAGE patterns of total rice malt protein polypeptides were shown in Fig.1. It was indicated that several peptides (lane 3-7) were in range of 21.5 kDa to lower than 10 kDa. Which was similar to the findings reported by Selamassakul et al. (2014). Compared with commercial rice protein hydrolysate Oryza70 in the same concentration (lane 7 and 8), the SDS-PAGE profile of rice malt protein polypeptides which were darker than commercial products might be due to % protein in which rice malt hydrolysates (77.5%) were higher than commercial rice protein (70%). The electrophoretic patterns obtained from rice malt hydrolysates and commercial rice protein (Oryza70) illustrated clearly that each contained several similar components.

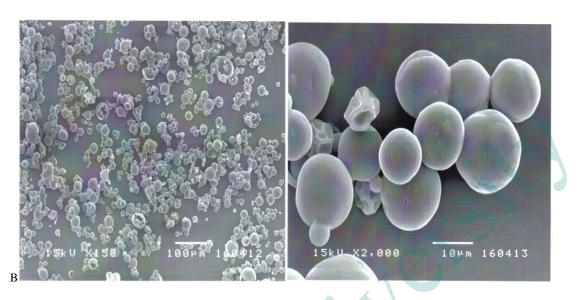
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**Figure 1** SDS-PAGE profile and molecular weight evaluation of protein hydrolysates from rice malt Lanes1-9:STD1, STD2,36µg RMP,27µg RMP,18µg RMP,9µg RMP, 1µg RMP,1µgOryza70, 10µg BSA

Characterization of particle morphology of rice malt peptide particles were examined by scanning electron microscope (SEM) with 150 and 2000 times magnification, compared to commercial soy peptide as shown in figure 2. The results indicated that rice malt peptide powder had good dispersion; therefore, it can easily dissolve in water, corresponding with its solubility of 92.71% as shown in Table 3. The small particle size is also beneficial to absorb easily into the body.





**Figure 2** Particle morphology of commercial soy protein hydrolysate powder (A) and rice malt protein hydrolysate powder (B) by SEM at 150x (left) and 2,000x (right) magnification.

For shelf life study, it was found that during storage at ambient temperature (AT), 25 °C and 4 °C for 5 years, The functional properties and physical qualities except for color value had not changed. This might be due to the fact that the higher amount of degradation products (amino acids and sugars) was attached to the maillard reaction thereby increased the reddish-brown color. While the antioxidative activities of protein hydrolysate slightly decreased as the storage time increased. The decreasion was more pronounced at ambient temperature and 25 °C, compared with 4 °C. The result of shelf life was shown in Table 4-6 and Figure 3.

Storage	Color			Conte	nts*		
Temperature		year 0	year 1	year 2	year 3	year 4	year 5
AT	L*	62.05 <sup>Ba</sup> ±0.33	62.05 <sup>Ba</sup> ±0.23	62.60 <sup>Aa</sup> ±0.10	62.70 <sup>Aa</sup> ±0.26	62.60 <sup>Aa</sup> ±0.22	62.70 <sup>Aa</sup> ±0.24
	a*	$2.77^{\text{Aa}} \pm 0.05$	$2.77^{\text{Aa}} \pm 0.03$	$2.20^{Ca} \pm 0.20$	$2.25^{CBa}\pm0.17$	$2.40^{\text{CBa}}\pm0.20$	$2.55^{\text{BAb}} \pm 0.21$
	b*	8.92 <sup>Ca</sup> ±0.19	$8.92^{Ca} \pm 0.19$	$8.90^{Ca}{\pm}0.10$	$9.12^{Ca}{\pm}0.08$	$9.80^{\text{Aa}} \pm 0.13$	$9.50^{Ba} \pm 0.10$
25	L*	62.05 <sup>Aa</sup> ±0.33	62.05 <sup>Aa</sup> ±0.23	62.23 <sup>Aa</sup> ±0.49	62.62 Aa ±0.32	62.35 <sup>Aa</sup> ±0.35	62.70 <sup>Aa</sup> ±0.45
	a*	$2.77^{\text{Aa}} \pm 0.05$	$2.77^{{}^{ABa}}\!\!\pm\!\!0.19$	$2.03 \overset{\text{Cab}}{=} \pm 0.06$	$2.50^{Ba} \pm 0.22$	2.62 <sup>Ba</sup> ±0.03	$2.76^{ABa} \pm 0.32$
	b*	$8.92^{\text{Ca}}\pm0.19$	$8.92^{{\rm CBa}} {\pm} 0.03$	$8.77^{Ca} \pm 0.06$	$8.98^{\operatorname{Bab}}{\pm}0.14$	$9.35^{\text{Aa}}\pm0.17$	9.30 <sup>Aa</sup> ±0.12
4	L*	62.05 <sup>Aa</sup> ±0.33	62.05 <sup>Aa</sup> ±0.23	62.00 <sup>Aa</sup> ±0.30	62.58 Aa ±0.32	62.57 <sup>Aa</sup> ±0.16	62.10 <sup>Aa</sup> ±0.20
	a*	$2.77^{\text{Aa}} \pm 0.05$	$2.77^{\text{Aa}} \pm 0.03$	$2.37^{\text{Ba}}\pm 0.25$	$2.37 {}^{\text{Ba}}\pm 0.32$	$2.65^{ABa} \pm 0.23$	$2.55^{ABb} \pm 0.18$
	b*	$8.92^{Ba} \pm 0.19$	8.92 <sup>Ba</sup> ±0.19	$8.90^{\text{Ba}}\pm0.00$	$8.92^{\text{Ba}}\pm0.03$	9.47 <sup>Aa</sup> ±0.38	9.35 <sup>Aa</sup> ±0.43

Table 4 Changes in color of protein hydrolysate from rice malt stored at AT, 25 and 4°C for different times

\*Means of triplicates ± standard deviation of mean

Different superscripts (A,B,..) in the same row indicate significant differences (p<0.05)

Different superscripts (a,b,..) in the same column indicate significant differences (p<0.05)

Items	Storage	Contents*					
	Temperature	Year o	Year 1	Year 2	Year 3	Year 4	Year 5
Moisture	AT	3.65 <sup>D</sup> ±0.04	3.63 <sup>D</sup> ±0.00	4.32 <sup>Ab</sup> ±0.00	4.07 <sup>C</sup> ±0.00	3.58 <sup>D</sup> ±0.00	4.22 <sup>Ba</sup> ±0.04
(%)	25	$3.65^{D} \pm 0.04$	$3.63^{D} \pm 0.00$	5.09 <sup>Aa</sup> ±0.01	$4.10^{B}\pm0.00$	$3.33^{D} \pm 0.00$	4.03 <sup>Ba</sup> ±0.10
	4	$3.65^{\text{BC}}{\pm}0.04$	$3.63^{BC} \pm 0.00$	$4.36^{AC} \pm 0.00$	$4.14^{\text{AB}}{\pm}0.00$	$3.09^{C} \pm 0.00$	3.45 <sup>СВь</sup> ±0.35
Water activity	AT	$0.28^{Ba} \pm 0.05$	0.28 <sup>Ba</sup> ±0.01	$0.28^{Bb} \pm 0.01$	$0.28^{Ba} \pm 0.00$	0.31 <sup>Aa</sup> ±0.01	$0.28^{Ba} \pm 0.01$
	25	$0.28^{\text{Ba}}{\pm}0.05$	$0.28^{\text{Ba}}{\pm}0.01$	$0.30^{Aa} \pm 0.00$	$0.28^{\text{Ba}} {\pm} 0.00$	0.29 <sup>Aa</sup> ±0.01	$0.29^{\text{Ba}}{\pm}0.01$
	4	$0.28^{Aa} \pm 0.05$	$0.28^{Aa} \pm 0.01$	$0.28^{\text{Ab}}{\pm}0.01$	$0.28^{\text{Aa}}{\pm}0.01$	0.29 <sup>Aa</sup> ±0.01	$0.029^{Aa} \pm 0.00$

Table 5 Changes in moisture	re, water activity and solubility contents of protein hydrolysate from rice malt	
stored at AT, 25 a	nd 4°C for different times	

\*Means of triplicates ± standard deviation of mean

Different superscripts (A,B,..) in the same row indicate significant differences (p<0.05)

Different superscripts (a,b,..) in the same column indicate significant differences (p<0.05)

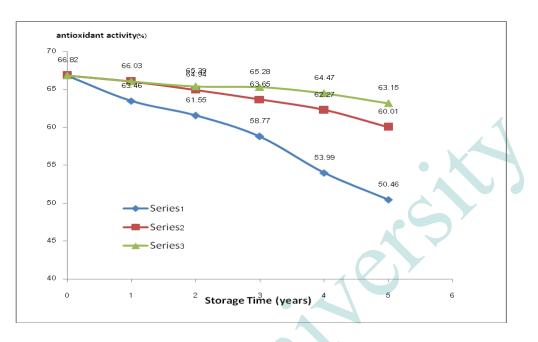
 Table 6
 Changes in functional properties of protein hydrolysate from rice malt stored at AT, 25 and 4°C for different times

unies								
Items	Storage Temperature		Contents*					
	remperature	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5	
Solubility	AT	92.75 <sup>Aa</sup> ±0.50	92.71 <sup>Aa</sup> ±0.47	92.48 <sup>Aa</sup> ±0.33	92.73 <sup>Aa</sup> ±0.15	92.79 <sup>Aa</sup> ±0.40	92.59 <sup>Aa</sup> ±0.36	
(%)	25	$92.75^{Aa} \pm 0.50$	92.71 <sup>Aa</sup> ±0.47	$92.72^{Aa} \pm 0.08$	$92.74^{\rm Aa} {\pm} 0.08$	$92.79^{Aa}\pm 0.38$	$92.76^{Aa}\!\!\pm\!\!0.27$	
	4	92.75 <sup>Aa</sup> ±0.50	92.71 <sup>Aa</sup> ±0.47	92.41 <sup>Aa</sup> ±0.06	$92.33^{Ab} \pm 0.14$	92.66 <sup>Aa</sup> ±0.12	$92.54^{Aa}\!\!\pm\!\!0.18$	
Foaming ability	AT	45.35 <sup>Aa</sup> ±1.12	46.10 <sup>Aa</sup> ±2.15	45.55 <sup>Aa</sup> ±1.35	46.34 <sup>Aa</sup> ±2.55	46.11 <sup>Aa</sup> ±1.55	46.12 <sup>Aa</sup> ±1.17	
(%)	25	45.35 <sup>Aa</sup> ±1.12	46.24 <sup>Aa</sup> ±1.97	46.55 <sup>Aa</sup> ±2.55	$46.50^{Aa} \pm 2.55$	$45.84^{Aa}\pm 2.15$	$46.24^{Aa} \pm 2.00$	
	4	45.35 <sup>Aa</sup> ±1.12	46.00 <sup>Aa</sup> ±2.45	46.10 <sup>Aa</sup> ±1.85	45.40 <sup>Aa</sup> ±1.55	45.72 <sup>Aa</sup> ±2.35	45.60 <sup>Aa</sup> ±2.10	
Emulsion activity	AT	$10.50^{Aa} \pm 0.85$	$11.55^{Aa} \pm 0.60$	$11.40^{Aa} \pm 0.92$	$10.74^{Aa} \pm 0.86$	$11.00^{Aa} \pm 0.72$	$10.65^{Aa} \pm 0.93$	
index (m²/g)	25	$10.50^{Aa} \pm 0.85$	11.62 <sup>Aa</sup> ±0.86	$11.62^{\rm Aa}\!\pm\!0.75$	$11.45^{Aa} \pm 0.26$	$11.15^{Aa} \pm 0.65$	$11.10^{Aa} \pm 0.70$	
	4	10.50 <sup>Aa</sup> ±0.85	$11.65^{Aa} \pm 0.70$	11.55 <sup>Aa</sup> ±0.86	11.15 <sup>Aa</sup> ±0.94	11.10 <sup>Aa</sup> ±0.55	11.15 <sup>Aa</sup> ±0.40	

\*Means of triplicates ± standard deviation of mean

Different superscripts (A,B,..) in the same row indicate significant differences (p<0.05)

Different superscripts (a,b,..) in the same column indicate significant differences (p<0.05)



**Figure 3** Changes in antioxidant activity of protein hydrolysate during storage at 4°C, 25°C and ambient temperature for 5 years.

From Figure 3, decreasing in antioxidant activity of protein hydrolysate under three storage temperature was observed. It was found that antioxidant activity decreased in all temperature. It was obviously observed that at 4 °C antioxidant activity showed slightly lower than 25°C and ambient temperature, respectively. This result is supported by the report by Yowapa et al. (2007). From Yaowapa et al. (2007) results, protein hydrolysate was more stable when stored at 4°C than 25°C. This might be due to the destruction of antioxidative compounds as the storage time increased, leading to some losses of antioxidative activity.

### 5. Conclusion

Protein hydrolysate from rice malt produced by Tipco biotech, Ltd. was composed of 77.5% protein, 143.15 mg/100 g phenolic compound, 12. 42% FAN and high level contents of vitamin B1, B3, B5 and B6 as Thai RDI. The composition of the amino acids in protein hydrolysates from rice malt was similar to protein hydrolysates from soybean and had the levels of all essential amino acids met the standard for the child and adult intake recommendation of FAO/WHO. Protein hydrolysate had excellent solubility at 92.71 %, and had foaming property as well as emulsifying property at 45.35% and 10.50 m<sup>2</sup>/g, respectively. It was noticeable that 5 years of storage time and temperature had no effect on physical, chemical and functional properties except for antioxidant activity. The shelf life evaluation revealed that the suitable temperature for storage protein hydrolysate was 4°C, followed by 25°C and ambient temperature, respectively. In conclusion, the rice malt protein hydrolysates are available and alternative materials with health-benefiting bioactivities for food ingredients in various food products.

## 6. Acknowledgements

This study was financially supported by the NRRL and ARDA. The author would like to thank the Tipco Biotech Co., Ltd. Prachupkirikhan for the raw materials provided.

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