Original Article

Determination of Monosodium-L-Glutamate in Chilled Ready Meals by Modified-High Performance Liquid Chromatography Method

Em-on Chaiprateep[1]* and Warachate Khobjai[1]

Abstract

Nowadays, most city families choose chilled ready meals instead of cooking their own meals because it is more convenient. Monosodium-l-glutamate (MSG) is a popular food additive used for enhancing the taste of foods. MSG should not be consumed more than 5 g/day because it can increase risk of metabolic syndrome such as hypertension, diabetes mellitus, dyslipidaemia and cardiovascular disease. However, nutrition label on ready meals does not show the amount of MSG. Therefore, this research was to determine the amount of MSG in chilled ready meals by modified-HPLC with UV detection at 210 nm. The method was derivatization of MSG by di-nitro-fluorobenzene (DNFB), using eclipse C18 column, mobile phase compose of methanol:water (50:50), flow rate 0.6 ml/min with run time 10 min. The results showed that the content of MSG in 27 samples separated in three groups including fried rice dishes (n=6), a la cart (n=15), spaghettis/noodle dishes (n=6), were 1.4±2.0 g. (0.01-7.33 g) per serving size. The amount of MSG found in the samples were not too high. However, if people consume chilled food from convenience store more than two times a day, they will receive 1,605 mg of sodium per day and 12 g of sugar per day which is beyond the amount recommended by the Department of Health, Ministry of Public Health. Therefore, cooking meal by ourselves with freshly prepared ingredients is the best way to decrease MSG, sodium and sugar exposure.

Keywords: *MSG, Chilled Ready Meals, HPLC, NCDs, Nutrition Label*

นิพนธ์ต้นฉบับ

การวิเคราะห์หาปริมาณผงชูรส (Monosodium-L-glutamate) ในผลิตภัณฑ์อาหารแช่เย็นสำเร็จรูป โดยวิธี Modified-High Performance Liquid Chromatography

เอมอร ชัยประทีป[1]* และวรเชษฐ์ ขอบใจ[2]

ผู้รับผิดชอบบทความ
(โทร.: 080-0666091,
e-mail: emon_c@rmutt.ac.th)
(1) วิทยาลัยการแพทย์แผนไทย
มหาวิทยาลัยเทคโนโลยีราชมงคลธัญบุรี,
ปทุมธานี, ประเทศไทย

บทคัดย่อ

ปัจจุบันผู้คนทั่วโลกนิยมเลือกบริโภคอาหารพร้อมรับประทานในกลุ่มอาหารแช่เย็นแทนการประกอบอาหารรับประทานเองที่บ้านเนื่องจากความสะดวกสบาย ผงชูรสเป็นสารปรุงแต่งเพิ่มรสชาติอาหารที่ได้รับความนิยม แต่ไม่ควรบริโภคผงชูรสเกินวันละ 5 กรัม เนื่องจากมีการศึกษาพบว่าสามารถเพิ่มความเสี่ยงในการเกิดโรคทางเมตาบอลิกได้ ได้แก่ ความดันโลหิตสูง เบาหวาน ไขมันในเลือดสูง และโรคหัวใจและหลอดเลือด ฉลากโภชนาการไม่ได้ระบุปริมาณผงชูรสในผลิตภัณฑ์อาหารสำเร็จรูป งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อวัดปริมาณผงชูรสในอาหารแช่เย็นสำเร็จรูปจำนวนรวม 27 ตัวอย่าง โดยวิธี Modified-High Performance Liquid Chromatography (Modified-HPLC) ด้วยตัวรับสัญญาณยูวีที่ความยาวคลื่น 210 นาโนเมตร โดยทำการเตรียมตัวอย่างเพื่อวิเคราะห์หาผงชูรสด้วย di-nitro-fluorobenzene (DNFB) ใช้คอลัมน์ eclipse C18 วัฏภาคเคลื่อนที่คือเมทานอลต่อน้ำ (50:50) อัตราเร็ว 0.6 มิลลิลิตรต่อนาที นาน 10 นาที ผลการศึกษาพบปริมาณผงชูรสเฉลี่ยต่อหนึ่งหน่วยบริโภคในอาหารแช่เย็นสำเร็จรูป ที่แบ่งออกเป็น 3 กลุ่ม คือ ข้าวผัด (6 ตัวอย่าง) อาหารจานเดียว (15 ตัวอย่าง) และอาหารจำพวกเส้น (6 ตัวอย่าง) คือ 1.4±2.0 กรัม (0.01-7.33 กรัม) ซึ่งปริมาณผงชูรสในตัวอย่างอาหารแช่เย็นต่อหนึ่งหน่วยบริโภคนั้นไม่สูงมากนัก อย่างไรก็ตามผู้บริโภคไม่ควรเลือกบริโภคอาหารแช่เย็นในร้านสะดวกซื้อเกินวันละ 2 มื้อ เนื่องจากจะได้รับโซเดียมวันละ 1,605 มิลลิกรัม และน้ำตาลวันละ 12 กรัม ซึ่งเกินกว่าปริมาณที่กรมอนามัยกระทรวงสาธารณสุขกำหนด ดังนั้น วิธีที่ดีที่สุดในการหลีกเลี่ยงผงชูรส เกลือ และน้ำตาล คือ การประกอบอาหารรับประทานเอง

คำสำคัญ: ผงชูรส, อาหารแช่เย็น, HPLC, ฉลากโภชนาการ

Introduction

Non communicable diseases (NCDs) composed of cardiovascular diseases (CVDs), cancer, chronic respiratory diseases, hypertension, obesity and diabetes are the major cause of morbidity and mortality than other causes combined in Thailand and globally (Bureau of Non Communicable Disease, 2017). NCDs mortality are increased from 38 million in 2012 to 52 million in 2030 (WHO, 2014). World health organization (WHO) has been estimated that the economic losses US$ 170 billion due to NCDs interventions in all low- and middle-income countries during 2011–2025 (US$1-3 per capita) (WHO, 2014). In 2025, the global agenda target that involved to nutrient on control and prevention NCDs are a 30% reduction in mean population intake sodium/salt, a 25% reduction in the prevalence of raised blood pressure or contain the prevalence of raise blood pressure, according to national circumstances and a 25% reduction in the overall mortality from CVDs, cancer, diabetes or chronic respiratory diseases (WHO, 2014). WHO recommends to intake a salt should less than 5 g per day (2,000 mg per day of sodium) to reduce blood pressure and coronary heart disease and stroke, because of nowadays people take excess sodium deaths from cardiovascular disease 1.7 million each year (WHO, 2014). The main source of salt is ready-meals (chilled and frozen ready meals) and processed foods (U.S. Food and drug administration [U.S. FDA], 2016). The survey in 2007 from ACNielsen reported that Thailand is the top ten countries favoured to consume ready meal. The two reasons that Thai population always should chilled food/frozen ready meals are the convenience to consume (no time to prepare) and cheaper price compared to cooking by themselves (Nielsen Thailand, 2016). Thai people consume salts with an average of 10.8 g/day. This amount is more than two times of salts recommended, which is 2,000 mg/day of sodium. WHO recommended consumer should intake sugar less than 25 g/day for improving quality of life (Thai FDA, 2017).

Many foods do not taste salty although they can be still high in sodium. Ready-meal and processed foods contain many ingredients with high amount of sodium such as monosodium glutamate (MSG), sodium bicarbonate (baking soda), sodium nitrite and sodium benzoate (U.S. FDA, 2016). MSG is a flavour enhancer that is well liked addition to food products for improved taste sensation. MSG consumption may be associated with increased body mass index to increased risk of overweight independent of physical activity and total energy intake in 752 healthy Chinese people (He et al., 2008). MSG should not be consumed more than 10 g per day because of it can increase neural defect like as Alzheimer, Parkinson and multiple system of brain atrophy (Em-on, 2015). MSG intake in 4 mg/kg for 3 weeks in rats (n=7) significantly ($p<0.05$) impaired short-term memory, long term memory, recognition memory and

decreased tryptophan in brain and plasma when compared to control rats that were injected with saline (1 ml/kg) (Khaliq et al., 2015). The study in 324 subjects in rural area of Thailand showed that the subjects' intake MSG 5 g/day for 10 days significantly increase metabolic syndrome incidence and overweight. Every 1 g of MSG intake significantly increased the risk of metabolic syndrome 1.14 fold (Khaliq et al., 2015). Thai health promotion foundation promotes how to decrease amount of sodium by keeping away seasoning sauce, fish sauce, fermented food, and MSG in cooking. When eating out at a restaurant, order for low MSG, sodium dishes. However, MSG has health benefit as it can improve palatability of food, influence the increase of appetite and replace salt in elderly people (Insawang et al., 2012). Although MSG does not show on the nutritional label, therefor the main point is check the nutritional facts label on sodium and MSG content to avoid high amount of sodium products,. This research aimed to determination of actual MSG content in chilled ready meals to use for the data on consumer's perception.

Research methodology

Experimental

● **Sample and Reagent**

The reference standard MSG was acquired from Sigma-Aldrich, USA. Methanol, water used were of HPLC grade, 2, 4, dinitro-1-fluorobenzene (DNFB), sodium bicarbonate, hydrochloric acid, diethyether were supplied by TTK Sciences. Thirty chilled ready meal samples purchased from the 2 convenience stores.

● **Standard and Sample Preparation**

The reference standard MSG was prepared in deionized water. The stock solution was serially diluted to make the working standard solution to obtain 0.125, 1.0, 2.5 mg/ml of MSG to make calibration curve (Figure. 1). Twenty-seven chilled ready meal samples were collected random samples from 4 brands in convenience store. Samples were separated to 3 groups 1) fired rice 6 samples 2) a la cart 15 samples and 3) spaghettis/noodle 6 samples. All samples were weighed to 50±2 mg in 100 ml volumetric flask of deionized water and then filtered by filter paper No. 1. The filtrates containing isolated MSG were collected and pH was adjusted to 7.8. Both the reference standard MSG and samples needed pre-column derivatization technique involving a reaction between a derivatizing agents by DNFB before injection onto the column. Aliquot of 0.5 ml of standard and samples were transferred to a test tube and 10 μl of DNFB was added. The mixture solution was shaken in the dark water bath at 40°C for 3 hrs. After that the mixture solution was acidified with 50 μl of HCL (6 M) and excess of DNFB was removed by extracting with 1 ml of diethyl ether. The extraction continued until the ether no longer gave color. The ether were evaporated and the leftover residue was collected with 500 μl methanol out of

which 50 µl of each sample and standard was injected for analysis (Lateef, 2012).

- **Instrumentation and HPLC analysis**

Agilent LC 1260HPLC equipped were running with 210 nm UV detector. Reversed phase C18 analytical column (ZORBAX Eclipse Plus, 5 µm, 4.6x100 mm) was used. Standard and samples were sonicated by sonicator and pH was adjusted by digital pH meter (Mettler Toledo^{TM}). Mobile phase consisted of methanol:water (1:1), flow rate of 1.6 ml/min, injection volume 50 µl with column temperature 25°C

- **Limit of Quantitation, LOQ**

LOQ = 10σ/S

σ = the standard deviation of the response

S = the slope of the calibration curve

Results and Discussion

MSG content per serving in twenty-seven chilled ready meal samples was 1.4±2.0 g. (0.01-7.33 g) (Table 1, Figure 2) and LOQ was 2.37 mg/ml. MSG per 1 serving size of chilled samples is not over dose that can cause health problem. However, consumer should be aware of the sodium and sugar content in chilled ready meal samples which were higher; 986.7±313.8 mg (530-1,480 mg) and 11.5±4.8 g (0-19 g), respectively, whereas fibre content was low; 3.4±0.9 g (0-4 g). Therefore, consumers should not consume more than 2 meals of chilled ready meals per day to avoid consuming too much sodium and sugar as recommended. The important point from nutrition label showed that some chilled ready meals such as sample number 22 and 27 contain low energy but high in sodium and sugar content. Therefore, consumer should pay attention to nutrition labeling in food products before making decision to buy them. According to WHO, recommended daily consumption of sodium is less than 2,000 mg, and less than 25 g of sugar, while daily consumption of fibre is more than 25 g. Following the recommended consumption can decrease metabolic syndrome and increase good quality of life. The best way to do is cooking meal ourselves and control the content of salt/sodium adding to foods when cooking and eating. Mix flavor foods like herbs, spices and no salt seasoning instead (U.S. FDA, 2016). WHO tries to diverse setting and make use of all available tools such as labeling, legislation, product reformulation, fiscal incentives that encourage the production and consumption of foods with reduced sodium content and education to consumer on nutrition therapy (WHO, 2014). Thai FDA launched a campaign called "Healthier Choice". The healthier choice symbol indicates healthier food products that decreasing sugar, salt and fat for improving quality of life and decreasing progression to NCDs (CVDs, cancer, diabetes, chronic respiratory diseases).

Conclusion

Although MSG is not shown on the nutritional label and MSG content per serving in chilled ready meal samples are not over recommended amount, consumer can use the Thai food and drug administration's guideline daily amounts (GDA) that provides the lists of energy, sodium, sugar and fat contained in a serving size on nutrition labelling for consumer's decision. Consumer can avoid MSG exposure by decreasing eating out/ buying food from restaurant. Meanwhile, try to increase cooking meal our-self with freshly prepared meal and decrease condiment in our dishes.

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Table. 1: Nutrition labelling and MSG content in 27 chilled ready meals

Sample	Name	Type*	Total weight (g/serving)	Energy (Kcal)	Sugar (g)	Fat (g)	Sodium (mg)	CHO (g)	Fibre (g)	Protein (g)	MSG (g/serving)
1	Stir Fried Rice mixed Minced Pork Basil and Omelets	1	205	400	6	11	1240	62	-	12	0.39
2	Pork Fried Rice	1	180	330	3	8	780	54	-	10	5.62
3	Tom Yum Fried Rice with Tenderized Pork	1	215	-	-	-	-	-	-	-	1.75
4	Stewed Pork Leg on Rice	1	265	380	8	9	570	45	-	-	1.18
5	Stir-Fried Basil Pork with Fried Rice	1	280	-	-	-	-	-	-	-	0.03
6	Kua Kling Pork with Rice & Boiled Egg	1	200	-	-	-	-	-	-	-	0.09
	Average±SD		224.2±35.7	370.0±36.1	5.7±2.5	9.3±1.5	863.3±342.7	58.0±5.7	-	11.0±1.4	1.51±2.1
7	Stir Fried Pork and Basil with Rice	2	185	330	2	12	630	80	2	10	6.34
8	Steamed Chicken with Rice	2	275	360	6	12	1040	67	2	15	2.84
9	Chicken Stir-Fried Chili	2	230	-	-	-	-	-	-	-	0.32
10	Shrimp Omelette with Rice	2	240	420	2	13	610	71	4	15	0.17
11	Fried Chicken with Garlic and Pepper with Rice	2	330	480	5	7	730	63	2	25	0.25
12	Grilled Pork Rice	2	58.85	440	2	12	530	60	2	-	0.01
13	Stir-Fried Chilli Fish with Rice	2	235	410	1	9	680	60	2	16	0.88
14	Stir-Fried Basil Pork	2	250	460	2	12	690	68	0	17	0.88
15	Stir-Fried Basil Chicken & Fried Egg with Rice	2	265	480	2	16	680	56	1	21	0.82
16	Spicy Minced Pork & Grilled Pork with Rice	2	230	430	3	14	830	48	1	15	0.99
17	Stir-Fried Chicken and Basil with Rice	2	220	390	0	10	530	-	-	14	1.13
18	Deep Fried Fish with Herb & Spicy Sauce with Rice	2	250	420	8	7	970	-	-	20	0.63
19	Charcoal-Grilled Chicken with Rice	2	205	320	8	4.5	640	-	-	15	0.05
20	Chicken with Sticky Rice	2	195	350	6	9	590	-	-	20	1.19
21	Pork Porridge	2	250	-	-	-	-	-	-	-	0.08
	Average±SD		226.3±60.3	406.9±53.8	3.6±2.7	10.6±3.2	703.8±156.4	60.5±10.9	1.8±1.1	16.9±3.9	1.1±1.6
22	Fried Noodle with Pork	3	180	300	12	7	750	53	-	7	7.33
23	Stir-Fried Soy Sauce Noodles with Pork	3	190	380	14	15	940	50	3	10	0.50
24	Chicken Spaghetti with Tomato Sauce	3	235	310	9	12	640	38	4	13	2.17
25	Stir-Fried Basil Chicken Spaghetti	3	250	460	10	15	1230	63	4	17	0.32
26	Fried Macaroni with Chicken	3	225	410	5	16	880	52	2	14	0.25
27	Spicy Vermicelli Salad	3	270	280	19	4.5	1480	48	4	11	0.49
	Average±SD		225±34.6	356.7±71.2	11.5±4.8	11.6±4.8	986.7±313.8	50.7±8.1	3.4±0.9	12±3.5	1.8±2.8
	Average±SD		226.4±48.7	388±59.4	6.0±4.7	10.7±3.5	802.7±254.7	57.1±10.3	2.3±1.2	14.8±4.4	1.4±2.0

*1 = Fired rice dishes, 2 = A la cart, 3 = Spaghetti/noodle dishes
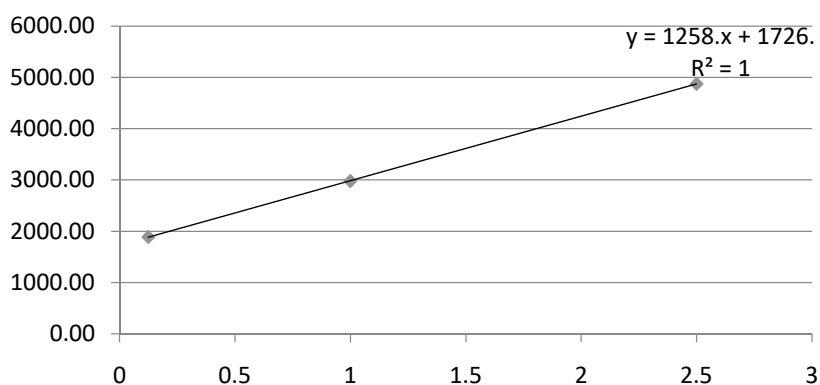
Figure 1 Calibration curve for MSG standards from 0.125, 1.0, 2.5 mg/ml with a correlation coefficient of 1.000
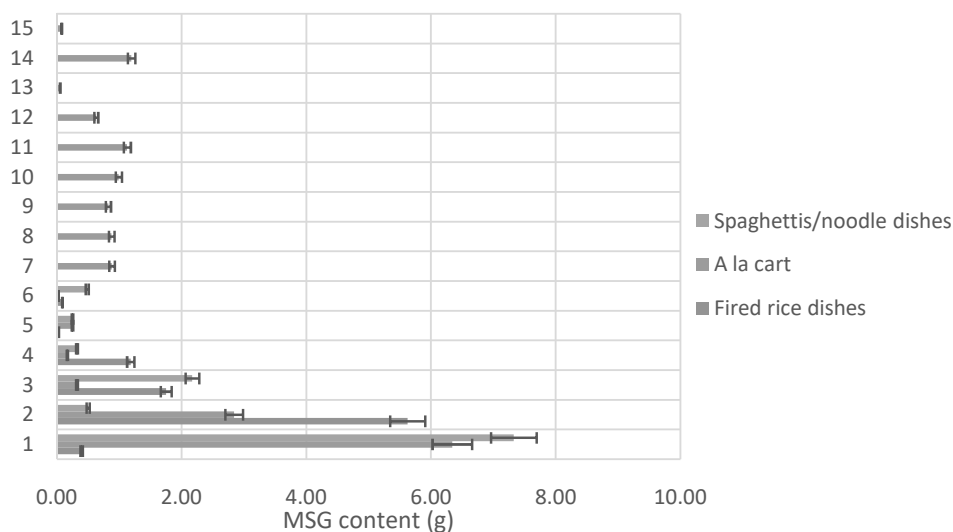
Figure 2 MSG content (g) in 3 groups of 27 chilled ready meals