

Effect of microwave-assisted extraction on curcuminoid from turmeric and application in germinate-coated rice

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Abstract

Curcuminoids from turmeric (*Curcuma longa*) provide the powerful bioactive activities, such as anti-inflammatory, antibacterial, antidepressant, antitumor and gastro protective properties. The objective of this research was to investigate the effect of optimize microwave-assisted extraction (MAE) on curcuminoid from turmeric. The experiments were conducted using varying microwave power ranging from 400 to 800 watt and extraction times of 1, 2, 3, 4 and 5 minutes using 2×5 factorial experiment. The microwave power and extraction time were affected on extract yield, total phenolic content, antioxidant activity (EC₅₀) and curcuminoids content. The extraction using the microwave power of 800 watt provided significantly higher curcuminoids, including; curcumin, demethoxycurcumin and bisdemethoxycurcumin, than that of 400 watt. The extended extraction time increased curcuminoid content then stable after 3 minutes. The optimum curcumin extraction conditions were 800 watt microwave power and 3 minutes extraction time. This condition of MAE could provide good qualities of extract; 21.00% yield, total phenolic content (161.38±3.56 mg GAE/g extract), EC₅₀ (32.34±0.97 µg/ml), curcumin (227.86±0.35 mg/g extract), demethoxycurcumin (136.26±0.56 mg/g extract) and bisdemethoxycurcumin (67.08±0.18 mg/g extract). Application of curcuminoid extract in germinate-coated rice varied curcuminoid extract at 0.5, 1.0, 1.5 and 2.0% w/v. The results showed that consumers liked coated rice with curcuminoid extract at 1.0% most. Therefore, the extraction of curcuminoid by using microwave are benefit in saving time and solvent ,and also provide high quality and efficiency of extraction.

Key Words: Tumeric, extract, Curcumin, Bisdemethoxycurcumin, Demethoxycurcumin

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1. Introduction

The turmeric (*Curcuma longa*) belongs to the *Zingiberaceae* family and distributes through out tropical and subtropical region of the world. Turmeric is a perennial plant with pulpy, tuberous roots with a tough brown skin and a deep orange flesh. Turmeric are used as a dietary spice, coloring agent in foods and textiles, and a treatment for a wide variety of ailments. It is widely used in traditional Indian medicine. Several studies confirmed that turmeric extracts have powerful biological activities, such as anti-inflammatory (Jurenka, 2009) antibacterial (De *et al.*, 2000), antidepressant, antitumor (Wilken *et al.*, 2001) and gastroprotective (Kulkarni *et al.*, 2010) properties. Owing to its antioxidant properties, turmeric has been widely accepted as one of the spices with the highest antioxidant activity (Wojdylo *et al.*, 2007). The most active compound of turmeric are curcuminoid which recognized as rich source of phenolic compounds. Curcuminoid consist of three different compounds ; curcumin (C) 60–80%, demethoxycurcumin (DMC) 15–30% and bisdemethoxycurcumin (BDMC) 2–6 %. It also has potential as a pharmaceutical expend since it possess antioxidant anti mutagenic and anti HIV properties and can reduce blood glucose (Due *et al.*, 2006) and LDL (Fan *et al.*, 2006). Curcumin is crystalline powder which obtained by solvent extraction of ground rhizomes of turmeric and purification of extract is done by crystallization. The curcumin is oil soluble pigment having a melting point of 174°C. It is stable at acidic pH but readily decompose at pH above neutral. It is light sensitive especially in solution but highly stable to heat. The most conventional extraction method of curcuminoid has been solid liquid extraction. As the heating process continues for long time, many solvents usages, the target molecules possibly decompose (Rojsitthisak *et al.*, 2005). The microwave assisted extraction (MAE) is another alternative for curcuminoid extraction. MAE method is widely used for extraction. The cell bursting phenomenon probably facilities the entry of solvent to soluble out the target compound, So lead to faster and efficient extraction. The objective of this research was to study the effect of microwave aid extraction on curcuminoid from turmeric and application in coated rice.

2. Materials and Methods

2.1 Raw material

Dried turmeric from Premium Food Co.,Ltd., Chiang Mai, was milled by hammer mill machine and sieved. Particle size of turmeric powder was at 1.2 mm. and stored in aluminum foil bags under vacuum. Turmeric powder was analyzed for total phenolic content, antioxidant capacity (EC₅₀) and curcuminoid content.

2.2 Microwave-assisted extraction of turmeric

For the extraction procedure, Turmeric powder 5 g was mixed with 95% ethanol (50 ml). The mixture was placed in the center of microwave oven using different power; 400 and 800 watts and different duration time 1, 2, 3, 4 and 5 mins. The extracts were filtered and concentrated by rotary evaporator. This study based on 2×5 factorial design as 10 treatments with 3 replications.

2.2.1 % Extract yield

Extract yield calculated by $\text{yield (\%)} = (W_1 \times 100) / W_2$

Whereas W_1 ; weight of extracts after evaporate and W_2 ; weight of turmeric.

2.2.2 Determination of total phenolics content (TPC)

Sample 200 μl were introduced into test tubes and mixed with 1.0 ml of Folin-Ciocalteu's reagent and 0.8 ml of Na_2CO_3 7.5%. The absorbance of samples was measured at 765 nm after incubating at room temperature for 30 min. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry material. (Kahionen *et al.*, 1999)

2.2.3 Determination of DPPH free radical scavenging activity

One milliliter of DPPH radical solution was mixed with 3 ml of methanolic solution of extracts. The reaction mixture was left for 30 min in dark room temperature. The absorbance was recorded at 517 nm. The concentration of antioxidant required for 50% scavenging of DPPH radicals (call EC_{50}) was calculated. (Singh *et al.*, 2010)

2.2.4 Determination of curcuminoid content

Agilent 1100 HPLC system with RESTEK Ultra C_{18} column (250×4.6 mm; 5 μm) was used to obtain the chromatograms. One percent of acetic acid in water (A) and acetonitrile (B) were used as mobile phases using the following profile : 0–20 min, 40%B ; 20–32 min, 60%B ; 32–38 min, 100%B ; 38–40 min, 40%B. The flow rate was 1.0 mL/min, the signals were observed at 425 nm.

2.3 Application of curcuminoid extract in germinate-coated rice

The preparation of germinated rice was conducted by using 500 g of brown rice and soaking in 1000 ml of water under temperature at 33°C for 300 minutes then germinating in incubator at 25°C for 24 hours. Germinated rice was cooked and dried in hot air oven at 133°C. (controlling moisture content under 14%).

The addition levels of turmeric extracted were at 0.5%, 1.0%, 1.5%, 2.0% w/w and 0% was a controlled treatment (without curcuminoid coating). Quantities of extract were coated on 100 g of germinated rice. Acacia gum and maltodextrin were used as coating material (Timabud *et al.*, 2010 and Singh, 2015).

2.3.1 Determination of curcuminoid content

Ten gram of sample was extracted with 100 ml of 95% (v/v) ethanol and extraction was in a shaker for 24 h. Agilent 1100 HPLC system with RESTEK Ultra C₁₈ column (250×4.6 mm; 5µm) was used to obtain the chromatograms. One percent of acetic acid in water (A) and acetonitrile (B) were used as mobile phases using the following profile : 0–20 min, 40%B ; 20–32 min, 60%B ; 32–38 min, 100%B ; 38–40 min, 40%B. The flow rate was 1.0 mL/min, the signals were observed at 425 nm.

2.3.2 Consumer acceptance Test

For the consumer test, 50 untrained consumers were recruited from Chiangmai University. The experiments were separated into 2 parts; testing the uncooked germinated brown rice coated with turmeric extracted and testing cooked germinated brown rice coated with turmeric extracted. The cooked sample was control at 50°C while testing. The 9-point hedonic scale, where in 1 = “dislike extremely”, 5 = “neither dislike nor like”, and 9 = “like extremely.” was used for all acceptability ratings, There were four formulations and one control formulation, each coded with 3-digit random number. Sensory acceptability of all attributes for each formulation was rated before proceeding to the next one.

2.4 Statistic analysis

Experimental results were the means±standard deviation of three parallel measurements. The results were analyzed by Factorial design using Design expert (Stat Ease Inc., Minneapolis) and SPSS version 17.0 program and $p \leq 0.05$ was considered as significant difference.

3. Results and Discussion

3.1 Chemical composition of turmeric powder

The major compositions of dried turmeric before extraction are shown in Table 1. Total phenolic were 35.72 mg/g, and antioxidant capacity (EC₅₀) were 89.14 µg/ml. The curcuminoid compounds were analyzed using HPLC (Fig.1) which curcuminoid content; curcumin, demethoxycurcumin and bisdemethoxycurcumin were 2.80%, 1.41% and 0.47%, respectively.

Table 1 Total phenolic content, EC₅₀ and curcuminoid contents of dried turmeric^a

Sample	Total phenolic content (TPC) (mg GAE / g db)	EC ₅₀ (µg/mL)	Curcumin (C) (%w/w)	Demethoxy- curcumin (DMC) (%w/w)	Bisdemethoxy- curcumin (BDMC) (%w/w)
Dried turmeric	35.72±2.45	89.14±4.33	2.80±1.7	1.41±0.78	0.47±1.04

Note: ^a value are the mean ± standard deviation (n=3).

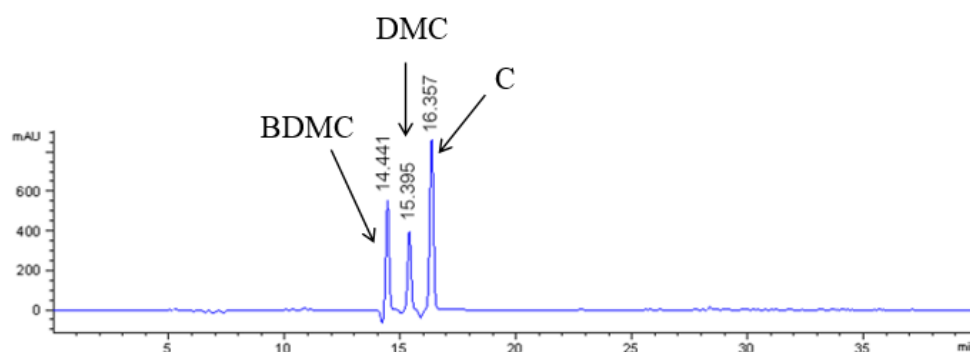


Figure1 Chromatogram of curcuminoids using HPLC

3.2 Effect of microwave-assisted extraction on chemical compounds of turmeric

Difference conditions of microwave power at 400 and 800 watts and different times for extraction at 1, 2, 3, 4 and 5 min of each treatment are shown in Table 2 and 3. There were significant difference of extract yield (%), total phenolic content, antioxidant capacity (EC_{50}), curcumin content; curcumin, demethoxycurcumin and bisdemethoxycurcumin.

Table 2 Extraction yield, total phenolic content and EC_{50} value of turmeric extracts with different duration of microwave powers and times

Microwave power (watt)	Time (min)	Extraction yield (%db) ^B	Total phenolic content (TPC) (mg GAE / g extract) ^C	EC_{50} (μ g/mL) ^C
400	1	21.20 $\pm$ 0.28 ^{Ae}	117.58 $\pm$ 0.45 ^d	52.24 $\pm$ 1.72 ^b
400	2	22.80 $\pm$ 0.28 ^{bc}	118.22 $\pm$ 0.89 ^d	52.97 $\pm$ 1.22 ^{bc}
400	3	23.00 $\pm$ 0.28 ^{bc}	119.49 $\pm$ 1.34 ^d	53.13 $\pm$ 0.69 ^{bc}
400	4	22.50 $\pm$ 0.71 ^{bcd}	109.25 $\pm$ 2.00 ^e	56.65 $\pm$ 1.14 ^{bc}
400	5	24.60 $\pm$ 0.57 ^a	51.60 $\pm$ 2.45 ^f	63.14 $\pm$ 1.61 ^{cd}
800	1	20.90 $\pm$ 0.14 ^e	131.30 $\pm$ 1.11 ^c	41.32 $\pm$ 0.62 ^a
800	2	22.20 $\pm$ 0.00 ^{cd}	140.12 $\pm$ 2.90 ^b	39.03 $\pm$ 0.43 ^a
800	3	21.00 $\pm$ 0.28 ^e	161.38 $\pm$ 3.56 ^a	32.34 $\pm$ 0.97 ^b
800	4	21.70 $\pm$ 0.14 ^{de}	45.78 $\pm$ 2.23 ^g	66.51 $\pm$ 1.96 ^{de}
800	5	23.10 $\pm$ 0.42 ^b	45.46 $\pm$ 4.01 ^g	63.66 $\pm$ 0.84 ^e

Note: A values are the mean $\pm$ standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

^B Dry weight basis of the original sample.

^C Calculated by using weight of the ethanolic plant extract.

Table 3 Curcuminoid content duration of microwave power and time^a

Microwave Power(watt)	Time (min)	Curcumin (C) (mg/g extract) ^B	Demethoxycurcumin (DMC) (mg/g extract) ^B	Bisdemethoxycurcumin (BDMC) (mg/g extract) ^B
400	1	127.36±1.45 ^A ^e	58.84±0.40 ^g	27.66±0.16 ^g
400	2	129.75±0.28 ^e	60.31±0.08 ^g	28.58±0.06 ^g
400	3	145.11±1.24 ^d	65.69±0.04 ^f	31.23±0.14 ^f
400	4	153.01±0.16 ^d	67.79±0.07 ^e	31.78±0.18 ^f
400	5	206.95±26.55 ^b	84.78±2.38 ^d	42.65±1.61 ^c
800	1	131.79±2.16 ^e	69.45±0.24 ^e	36.87±0.04 ^e
800	2	182.32±4.11 ^c	83.63±0.66 ^d	42.17±0.46 ^{cd}
800	3	227.86±0.35 ^a	136.26±0.56 ^a	67.08±0.18 ^a
800	4	203.01±9.70 ^b	107.64±0.66 ^b	41.13±0.91 ^d
800	5	198.33±7.18 ^b	100.13±0.90 ^c	55.70±0.45 ^b

Note: A value are the mean±standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

^B Calculated by using weight of the ethanolic plant extract.

Extraction yield using microwave power at 400 and 800 watts for extraction were 20–24% of dried extracts. It was higher than extraction by hydro distillation technique, low pressure solvent technique and supercritical fluid extraction that provided 3.1%, 14% and 12% yield, respectively (Mara *et al.*, 2006). Because the dry microwave irradiation can hydrolyze either linkages in cellulose and convert it into soluble fractions with in 1 or 2 min. The high temperature attained by the cell wall enhance degradation of cellulose reducing its mechanical strength. This disruption should increase the rate of extraction and efficiency of extraction in given time (Deepak *et al.*, 2002).

Total phenolic content of extract at power 400 watts was increased follow by extraction time but not significant difference ($p \geq 0.05$) then decreased in 4 min of extraction ($p \leq 0.05$). The highest total phenolic content was at 3 min extraction contained 119.49 mg/g extract. Also same as extraction at 800 watts; an increase of extraction time made higher total phenolic ($p \leq 0.05$) and went to the highest at 3 min contained 161.89 mg/g extract then decreased in 4 and 5 min of extraction time.

Trend of antioxidant capacity (EC_{50}) was as same as total phenolic content because the higher of total phenolic content indicated the high of antioxidant (Yang *et al.*, 2006). The highest antioxidant capacity (EC_{50}) at 400 watts were 52.24 mg/ml extracted in 1 min of extraction and 800 watts were 52.24 mg/ml extracted were 32.34 mg/ml extracted in 3 min of

extraction. Two hours of extraction time using solid liquid extraction technique provided total phenolic contents at 146.65 mg/g (Surojnametakul *et al.*, 2012) nearby microwave extraction at 800 watts 2 min of this experiment. The extraction at 800 watts 5 min gave total phenolic content close to Yadav and Kumar (2013) who extracted curcuminoid for 8 hours using enzymatic technique (31.00 mg/g extract). The highest antioxidant capacity (EC_{50}) using irradiation for curcuminoid extraction (Thongpasuk *et al.*, 2013) contained 65 mg/ml extracted nearby microwave extraction at 800 watts 4 min.

The curcuminoid content; curcumin, demethoxycurcumin and bisdemethoxycurcumin were significantly ($p \leq 0.05$) increase when microwave power increase and the highest at 5 min were 20.66, 8.48 and 4.27%, respectively, closed to Revety (2001) who extracted curcuminoid in methanol solvent using soxhlet technique for 6 hours gave curcumin (15.18%), demethoxycurcumin (9.90%) and bisdemethoxycurcumin (4.73 %).

Curcuminoid contents at 800 watts of extraction was significantly ($p \leq 0.05$) increased from 1 min and the highest in 3 min. The curde extract contained curcumin (22.79%), demethoxycurcumin (13.63%) and bisdemethoxycurcumin (6.71%), closed to Revety (2011) who got 22.8%, 14.2% and 6.5% of curcumin, demethoxycurcumin and bisdemethoxycurcumin.

Curcuminoid content duration of microwave power at 400, 800 watts are shown in Figure 2. Different power of extraction gave significantly ($p \leq 0.05$) different of three composition of curcuminoid content. An extraction at 800 watts contained overall curcuminoid content more than using 400 watts.

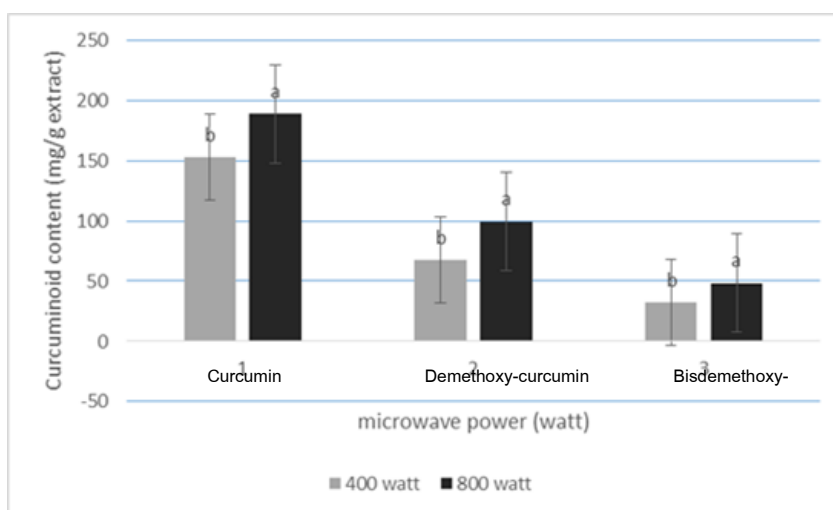


Figure 2 Effect of microwave power on curcumin (1), demethoxycurcumin (2) and bisdemethoxycurcumin content (3) of turmeric extract.

Deepak *et al.* (2000) reported that microwave-assisted extraction made the cork cells disturbed and the cell were swollen. The oleoresin cells were also swollen, sometimes with broken cell walls with the pigments scattered near the broken part of cell wall and outside. The disturbed cork cell and the broken oleoresin cells make curcuminoids more easily accessible to the solvent. Thus a prior microwave irradiation helped to increase extraction rates and also the suitable solvent and more power of microwave would increase curcuminoid contents.

Curcuminoid content by duration time for extraction at 1, 2, 3, 4 and 5 min are shown in Figure 3. An increasing of extraction time gave higher extract and stable similar to Wakte *et al.* (2011) that extract curcumin using microwave technique found that an increase time of extraction increased curcuminoid content and finally stable. Paulucci *et al.* (2012) said that an increase time for curcuminoid extraction gave lower efficiency because curcuminoid were decreased. Extraction of *Myrtus Communis L.* Leaves by using microwave technique by Dahmoune *et al.* (2014) found that an increase of extraction time increased the quantity of analyzed extract, although there was the risk of the degradation of extracted compounds.

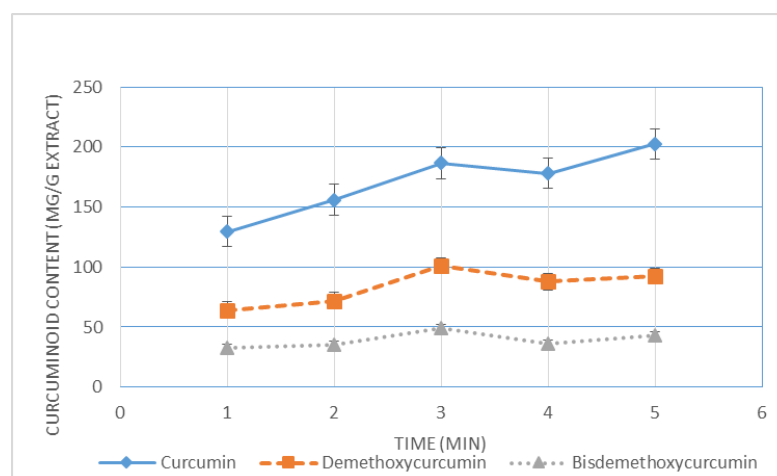


Figure 3 Effect of microwave extraction time on curcumin, demethoxycurcumin and bisdemethoxycurcumin content of turmeric extract

3.3 Application of curcuminoid extract in germinate-coated rice

As shown in table 4 and 5, L^* a^* b^* value and curcuminoid content were significantly different. L^* and a^* value were significantly decreased with adding more curcuminoid, while b^* value was significantly increased. It was indicated that the coated rice were get in dark. Curcuminoid content; curcumin, demethoxycurcumin and bisdemethoxycurcumin were significantly increased with addition more turmeric extract.

Table 4 Color; L* a* b*, and moisture content with addition of 0.5, 1.0, 1.5, 2.0 % and control of turmeric extracted in coated rice.*

Tumeric extracted addition (%)	L*	a*	b*	MC (ns)
0.5	57.21±0.06 ^a	9.23±0.08 ^c	31.57±0.40 ^a	8.31±0.07
1.0	55.25±0.05 ^b	11.41±0.08 ^b	30.54±1.00 ^b	8.31±0.11
1.5	54.15±0.13 ^c	11.48±0.06 ^{ab}	28.92±0.13 ^d	8.30±0.07
2.0	54.01±0.17 ^c	11.65±0.13 ^a	29.62±0.07 ^c	8.26±0.06
control	60.29±0.23	2.94±0.19	18.38±0.21	8.19±0.13

Note: * value are the mean ± standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

Table 5 Curcuminoid content with addition of 0.5, 1.0, 1.5, 2.0 and control of turmeric extracted in coated rice.*

Tumeric extracted addition (%)	Curcumin g/100g rice db	Demethoxy curcumin g/100g rice db	Bisdemethoxy curcumin g/100g rice db
0.5	0.0164±0.0003 ^d	0.0095±0.0004 ^d	0.0027±0.0006 ^d
1.0	0.0188±0.0001 ^c	0.0162±0.0004 ^c	0.0132±0.0005 ^c
1.5	0.0241±0.0004 ^b	0.0314±0.0002 ^b	0.0393±0.0007 ^b
2.0	0.0398±0.0003 ^a	0.0339±0.0007 ^a	0.0774±0.0005 ^a
control	-	-	-

Note: * value are the mean ± standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

The data of the sensory scores of uncooked samples are shown in table 6. It can be seen that the trends for sensory rating was increased at 1.0% and significantly decreased at 1.5% addition of curcuminoid. At 1.0% of curcuminoid addition, sensory liking were the highest for all attributes. The score was around 6.1–6.9, indicating that consumer “like slightly.” Also cooked rice (Table 7), at 1.0% addition, sensory score was the highest for all attributes. The score was around 6.0–7.1, indicating that consumer “like slightly” to “like moderately” and slightly decreased at 1.5% addition.

Table 6 Sensory scores of uncooked germinated instant rice coated with turmeric with addition of 0.5, 1.0, 1.5, 2.0% and control of turmeric extracted.*

Tumeric extracted addition (%)	Color	Appearance	Turmeric odor	Overall odor	Overall liking
0.5	6.4±1.2 ^{ab}	6.3±1.3 ^{ab}	6.4±0.5 ^a	6.7±0.9 ^a	6.4±1.0 ^a
1.0	6.9±1.1 ^a	6.7±1.0 ^a	6.3±1.1 ^a	6.3±0.7 ^b	6.1±0.7 ^{ab}
1.5	6.1±1.3 ^b	6.3±1.0 ^{ab}	5.9±1.3 ^b	6.1±1.3 ^b	6.0±0.8 ^b
2.0	5.6±1.0 ^c	5.8±1.1 ^b	5.6±1.2 ^b	5.7±1.2 ^c	5.7±1.2 ^c
control	6.2±0.8	6.4±0.5	5.6±1.4	6.4±0.9	5.9±1.2

Note: * value are the mean ± standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

Table 7. Sensory Scores of cooked germinated instant rice coated with turmeric with addition of 0.5, 1.0, 1.5, 2.0% and control of turmeric extracted.*

Tumeric extracted Addition (%)	Color	Appearance	Turmeric odor	Turmeric flavor	Taste	Tenderness ns	Overall liking
0.5	7.1±0.8 ^a	6.7±0.9 ^b	6.0±1.2 ^b	5.7±1.3 ^a	6.3±1.4 ^a	5.6±0.5	5.6±0.5 ^b
1.0	7.1±1.0 ^a	7.0±1.1 ^{ab}	7.0±1.1 ^a	6.1±0.6 ^a	6.1±0.8 ^{ab}	5.9±1.0	6.0±1.1 ^a
1.5	6.4±0.7 ^b	7.1±0.6 ^a	5.7±1.8 ^b	6.0±1.6 ^a	5.9±1.0 ^b	5.6±0.9	5.1±1.0 ^c
2.0	5.7±0.9 ^c	5.9±1.3 ^c	4.9±1.4 ^c	4.4±1.0 ^b	5.0±0.5 ^c	5.7±0.4	4.1±0.6 ^d
control	6.7±1.5	6.5±1.1	6.2±1.4	5.2±1.2	5.6±0.5	5.9±1.2	5.6±1.2

Note: * value are the mean ± standard deviation (n=3). Means within each column followed by a different letter are significantly different ($p \leq 0.05$).

4. Conclusion

Curcuminoid extraction using microwave-assisted extraction at 400 and 800 watts of microwave power and 1, 2, 3, 4 and 5 min for extraction time then analyzed extract yield (%), total phenolic content, antioxidant capacity (EC_{50}), curcuminoid content; curcumin, demethoxycurcumin and bisdemethoxycurcumin. The results found that the optimum condition was using microwave power at 800 watts for 3 min which provided high efficiency of curcuminoid and other bioactive compounds. Application of curcuminoid extract in germinated-coated rice at 1 % was the best sensory score and 78 % of consumers were accepted this product.

Acknowledgements

The authors would like to thank to acknowledge graduated school, Chiang Mai University and Lanna Rice Research Center, Chiang Mai University for financial support.

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