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Research Article

FORMULATION AND EVALUATION OF STABILITY OF THAI PURPLE RICE BRAN-BASED COSMETIC PRODUCTS

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ABSTRACT

Objective: The rice bran (RB) phytochemicals are known for several pharmacological properties. The current study was aimed to extract the active principles from Thai purple RB, formulate the cosmetic products, and evaluate the physical property and stability of the products.

Methods: Purple RB was extracted using 80% ethanol, and total phenolic and anthocyanin contents were measured using high-pressure liquid chromatography (HPLC) and spectrophotometric methods. Rice bran oil (RBO) was obtained by cold press method, and oryzanol content was determined using HPLC. The antioxidant capacity of RBO and purple rice bran extract (PRE) was assessed using spectrophotometric methods. The cosmetic products were formulated with PRE and fermented and distilled purple rice solution (FDPRS). RBO was also used as an ingredient in the formulation of solid soap. The stability of the formulations was also assessed. The heavy metal and microbial contamination were determined using atomic absorption spectrometry and plating assay, respectively.

Results: The yield of PRE and RBO was 0.92 and 1.62%, respectively. The total phenolic and anthocyanin content of PRE was 305.30±6.15 mg gallic acid/g of extract and 877.33±87.73 mg cyanidin chloride/g of extract, respectively. Oryzanol content of RBO was 6.33±0.09 mg/g of RBO. RBO, PRE, and FDPRS exhibited high antioxidant capacity. The cosmetic formulations were observed to be physically stable in the heat–cool cycle, and the stability of the active compounds was not affected while stored at 30°C for 2 months. The storage temperature affected the phenolic compounds present in the cosmetic formulations, especially at 45°C. There was no significant decrease in oryzanol content of soap formulation. All the formulations were free from microbial and heavy metal contamination.

Conclusion: The RB phytochemical-based cosmetic products are rich in antioxidants, stable, and free from heavy metals and microbial contamination, and further fine-tuning of the formulation may achieve the market quality.

Keywords: Antioxidants, Cosmetic, Thai purple rice, Rice bran, Rice bran oil, Heavy metals.

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INTRODUCTION

The cosmetic industries are massive, exclusively consumer-based business with the market value of about $11.7 billion as on 2016. Cosmetics are used to improve the physical appearance and prevent the natural processes that influence the exterior look of the human being, primarily, used against the aging process. Several cosmetic products claim that they have appropriate positive effects on the skin such as antiaging and skin tightening. The scientific reports and evidence about the prime compounds used in the cosmetic preparations are limited and capricious. Many cosmeceutical companies claim that they have used natural ingredients in their products [1].

Rice bran (RB) phytochemicals (flavonoids, phenolics, tannins, sterols, tocols, γ-oryzanols, and amino acids) are known for several pharmacological properties such as antioxidant [2], anti-colitis [3], anticancer, antitumor, antimutagenic [4], antidiabetes [5], ocular impairment [6], anti-aging [7], and anti-inflammatory [8].

Studies reported that rice bran extracts (RBE) are potent hair-growth inducer through 5-alpha-reductase inhibition without exhibiting any adverse effects [9,10]. Rice bran oil (RBO) is rich in unsaturated fatty acids (oleic, linoleic, and α-linolenic acids) and saturated fatty acids (palmitic and stearic acids). RBOs are used in the cosmetic products, mainly in sunscreen, antiaging, and skin-lightening products, because of its bioactivities and moisturizing properties on the skin [11-15]. Thai-pigmented rice has been reported as an active ingredient in cosmetics, pharmaceuticals, and functional foods [16]. The cosmetic surfactant derived from rice was proved as a potent emulsifier for making cosmetic products which consist of fatty ingredients [15].

Pengkumsri *et al.* [2] reported the phenolic acid content of RB (RB of black, red, and brown rice) extracted using 80% ethanol. Daud *et al.* [17] studied the impact of different solvent (hexane, ethanol, and methanol) extraction methods on total phenolic content and antioxidant properties of RBO of different rice varieties. Daud *et al.* [17] stated that total phenolic content and antioxidant activity depend on both solvent extraction method and RB types. Sukrasno *et al.* [18] reported that ethanol extract of black RB exhibited highest phytochemical content, namely, carotenoid, flavonoid, and phenolic content, and antioxidant activity when compared to that of the other extraction methods.

Pengkumsri *et al.* [19] reported the influence of extraction methods in the recovery of oryzanol and tocols in RBO of black, red, and brown rice. Hexane extraction showed a maximum recovery of oryzanol and tocols compared to that of the other extraction methods including cold press extraction method [19]. Cold press extraction was considered as a solvent-free and environmentally safe extraction method [20]. In the present study, the extracted RBO was used for the cosmetic formulation (solid soap). Therefore, a solvent-free technique was required for RBO extraction. Thus, the cold press extraction method was used to extract RBO.

Studies on the formulation and stability of the cosmetic products with natural ingredients are insufficient because of the trade secrets and marketing liability of the inventor or company. However, scientific reports on the cosmetic products are mandatory for the development of upgraded cosmetics with naturally available bioactive compounds. Thus, the current study aimed to extract the active principles from purple RB, formulate the cosmetic products, and evaluate the physical property and stability of the products.

METHODS

Raw materials

The purple rice was collected from local market of Chiang Mai, Thailand, and milled to acquire fresh RB (PRB). The fermented and distilled purple rice solution (FDPRS) was obtained from Health Innovation Institute, Chiang Mai, Thailand.

Extraction

RBO was extracted by the cold press method as described previously [19]. PRB was subjected to 80% ethanol extraction as described previously [2]. The extracts were stored at −20°C until analysis.

Assessment of oryzanol, phenolic, and anthocyanin content

Oryzanol content of RBO was determined by high-pressure liquid chromatography (HPLC) as detailed in the previous publication [19]. Total phenolic and anthocyanin contents of rice extract were analyzed using spectrophotometric methods. The quantification of each phenolic compounds was achieved by HPLC as detailed in the previous publication [2].

Determination of antioxidant capacity

Antioxidant capacity of RBO, PRE, and FDPRS was assessed by 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 1, 1-diphenyl-2-picryl-hydrazil (DPPH), and ferric reducing antioxidant power (FRAP), assays as described in our previous reports [2,21].

Formulations

The mineral spray, shampoo, liquid soap, and solid soap formulations were prepared as detailed in Table 1. The concentration of each compound is based on the data received from the Health Innovation Institute, Chiang Mai, Thailand (unpublished private data).

Stability study

Liquid or semi-solid cosmetic formulations such as shampoo, liquid soap, and mineral spray were subjected to cool–heat cycle process to check the stability. The samples were stored at 4±2°C for 48 h and then transferred to 45±2°C for 48 h. The cycling of cool and heat process was repeated for 6 times. Then, the changes (color and layer separation) in the samples were observed. In another experiment, formulations were stored at different temperatures (4±2, 30±2, and 45±2°C) for 2 months, and samples were collected every month for the quality evaluation. Color, fragrance, layer separation, and pH of the products were evaluated, and the total phenolic and oryzanol content of the formulas was measured to check the quality of the product.

Evaluation of heavy metal content

Lead, arsenic, mercury, and barium content in the cosmetic formulations were determined by atomic absorption spectrometry (Model-932 plus, GBC, Germany) as described in the previous publication [22,23].

Microbial contamination

The microbiological safety of the products was determined by evaluating the presence of representative microbial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* in the product by plate count method as detailed in the previous publication [24,25].

Statistical analysis

The report of the data was given as mean ± standard deviation. The Analysis of variance was performed using the statistical SPSS software version 17 (Chicago, SPSS Inc., U.S.A). Duncan's new multiple range tests determined significant differences, at the 95% confidential level ($p<0.05$).

RESULTS AND DISCUSSION

The PRE and RBO were prepared from fresh RB. The yield of PRE and RBO was 0.92 and 1.62%, respectively (Fig. 1a). Oryzanol content of RBO was observed to be 6.33±0.09 mg/g of RBO. The total phenolic and anthocyanin content of PRE was 305.30±6.15 mg gallic acid/g of extract and 877.33±87.73 mg cyanidin chloride/g of extract, respectively (Fig. 1b). The phenolic acids were determined by HPLC. Protocatechuic acid, caffeic acid, syringic acid, and *p*-coumaric acid in PRE were quantified, and the concentration was found to be 0.87±0.04, 1.02±0.05, 0.20±0.01, and 11.40±0.57 mg/g of extract, respectively (Fig. 1c). FDPRS was obtained from health innovation institute. Hence, the content of the FDPRS was not determined in the present study.

The antioxidant capacity of RBO was found as 6.25±0.21 mg trolox/g of RBO extract and 14.94±1.2 mg $FeSO_4$/g of RBO extract in ABTS and

Table 1: The composition of the cosmetic preparations

Mineral spray		Shampoo		Liquid soap		Solid soap	
Ingredient	Concentration (%)	Ingredient	Concentration (%)	Ingredient	Concentration (%)	Ingredient	Concentration (%)
DEB-96	10.00	Texapon® N8000	30.00	Texapon® N8000	26.00	Glycerin	90.00
Sodium benzoate	0.02	Texapon® N 40	10.00	Texapon® N 40	17.50	PRE	0.10
Sodium chloride	0.10	Comperlan® KD	2.00	Sodium lauryl sulfate	1.00	RBO	1.00
Copper sulfate	0.10	Glydant™	0.25	Comperlan® KD	2.00	FDPRS	3.00
Tween-80	0.50	Bronidox®	0.25	Allantoin	0.50	Water	5.90
PRE	0.10	10% EDTA	1.00	Glydant™	0.25		
FDPRS	10.00	Sodium chloride	2.00	Bronidox®	0.25		
Water	79.18	PRE	0.10	10% EDTA	1.00		
		FDPRS	10.0	Sodium chloride	2.00		
		Water	44.40	PRE	0.10		
				FDPRS	10.0		
				Water	39.40		

PRE: Purple rice bran extract, FDPRS: Fermented and distilled purple rice solution, RBO: Rice bran oil

FRAP assay, respectively. In DPPH assay, the IC_{50} concentration of RBO was 1.52±0.31 mg. The antioxidant capacity of PRE was determined as 24.68±1.30 mg trolox/g of PRE and 90.14±1.2 mg $FeSO_4$/g of PRE in ABTS and FRAP assay, respectively. The IC_{50} concentration of PRE was 0.71±0.20 mg in DPPH assay (Table 2). FDPRS showed free radical scavenging property (Table 2).

The cosmetic formulations were prepared as detailed in Table 1. Then, the formulations were subjected to stability study. The liquid and semi-liquid products were tested for the ability to withstand the drastic temperature changes by cool–heat cycle method. After three cycles of the cool-heat process, the color of the mineral spray, shampoo, and liquid soap becomes pale than that of its original color (red). The viscosity of the product was not altered, and no layer separation was observed (Table 3). The color of the product changed after three cycles of heating and cooling is due to the changes or deformation of the phytocompounds present in the formulas.

The formulations were stored at different temperatures (4, 30, and 45°C) for 2 months, and the changes were assessed. The color, fragrance, viscosity, and pH of the products were not changed, and no layer separation was detected (Table 4).

The degradation profile of active (phenolic) compounds of PRE was assessed when stored at a different temperature. The concentration of protocatechuic acid (0.87±0.04 to 0.91±0.03 mg/g extract), caffeic acid (1.01±0.05 to 0.97 ± 0.01 mg/g extract), and *p*-coumaric acid (11.40±0.57 to 11.09±0.53 mg/g extract) was not significantly altered during storage at 30°C for 2 months. There was no alteration observed in the concentration of syringic acid (0.19±0.01 mg/g extract) during storage at 30°C for 2 months (Table 5). The non-significant level of reduction in phenolic compounds was observed, which indicated that the PRE is stable for 2 months even when stored at 45°C.

The phenolic acid content of cosmetic products stored at various temperature was determined to evaluate the stability of active compounds in the cosmetic products. In mineral spray formulation, 29.47% degradation of phenolic compounds was observed during the storage period (2 months) at 45°C. In shampoo formulation, the

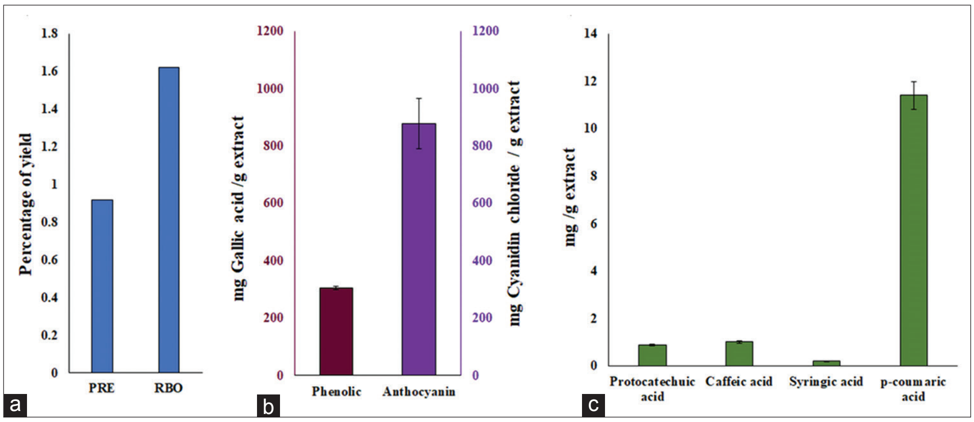

Fig. 1: The yield of purple rice bran extract (PRE) and rice bran oil (a), the phytochemical content of PRE (b), and phenolic acid composition (c) of PRE

Table 2: The antioxidant capacity of PRE, RBO, and FDPRS

Samples	DPPH assay (IC_{50})	ABTS assay (mg trolox/g or ml extracts)	FRAP assay (mg $FeSO_4$/g or ml extracts)
PRE	0.71±0.20 mg	24.68±1.30 mg/g	90.14±1.2 mg/g
RBO	1.52±0.31 mg	6.25±0.21 mg/g	14.94±1.2 mg/g
FDPRS	1.25±1.02 ml	0.01±0.00 mg/ml	0.07±0.00 mg/ml

PRE: Purple rice bran extract, FDPRS: Fermented and distilled purple rice solution, RBO: Rice bran oil, DPPH: 1, 1-diphenyl-2-picryl-hydrazil, ABTS: 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power

Table 3: The stability of the cosmetic formulations after cool–heat cycle study

Formulation	Color*		Viscosity**	Layer separation
	0–2 cycles	3–6 cycles	0–6 cycles	0–6 cycles
Mineral spray	Red	Lighter	+	No separation
Shampoo	Red	Lighter	++	No separation
Liquid soap	Red	Lighter	+++	No separation

*Color of the cosmetic formulations was red during 0–2 cycles and the color changed to lighter or pale after third cycle; **+: 0.69–0.72 mPa.s; ++: 990–999 mPa.s; +++: 2350–2450 mPa.s

Table 4: The stability of the products stored at 4, 30, and 45°C for 2 months

Formulation	Color	Fragrance	Viscosity	Layer separation	pH
Mineral spray	Red	Mild rice fragrance	+	No separation	5
Shampoo	Red	Mild rice fragrance	++	No separation	6
Liquid soap	Red	Mild rice fragrance	+++	No separation	6
Hard soap	Dark red	Mild rice fragrance	-	-	8

**+: 0.69–0.72 mPa.s; ++: 990–999mPa.s; +++: 2350–2450 mPa.s phenolic compounds were degraded up to 29.98% when stored at 45°C for 2 months (Fig. 2). In liquid soap formulation, degradation (30.03%) of phenolic compounds was observed when stored at 45°C for 2 months. Storage of hard soap at 45°C for 2 months results in the degradation (55.43%) of phenolic compounds (Fig. 3).

The phenolic compounds were observed to be stable in the liquid soap formulation when stored at 4°C and 30°C for 1-month storage duration and later showed reduction until the end of the experiment (Fig. 3). A significant level of reduction of phenolic compounds was observed in all the formulations while stored at 45°C for 2 months, whereas solid soap formulation showed a significant level of degradation (26.07%) of phenolic compounds while stored at 30°C for 2 months. The main active component of hard soap formula is oryzanol. Thus, the stability of oryzanol was determined and found that no degradation occurred until 15 days of storage at various temperature, whereas extended storage time displayed reduction (2.63, 3.62, and 6.25% degradation of oryzanol at 4, 30, and 45°C, respectively) in oryzanol content. The storage temperature (45°C) triggers the degradation of active compounds present in the cosmetic products (Fig. 4).

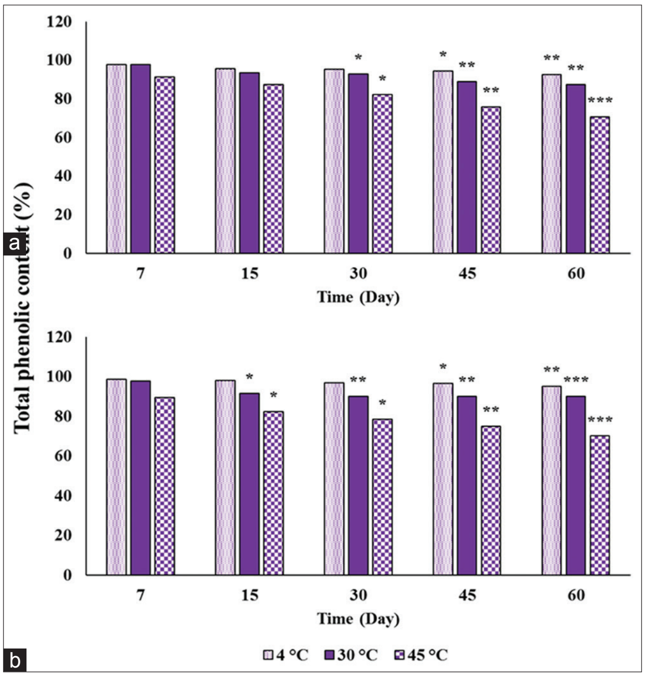

Fig. 2: The changes in the phenolic content of mineral spray (a) and shampoo (b) during storage at different temperature (4°C, 30°C, and 45°C). *, **, and * represent the significant variation with p>0.05, 0.01, and 0.001, respectively**

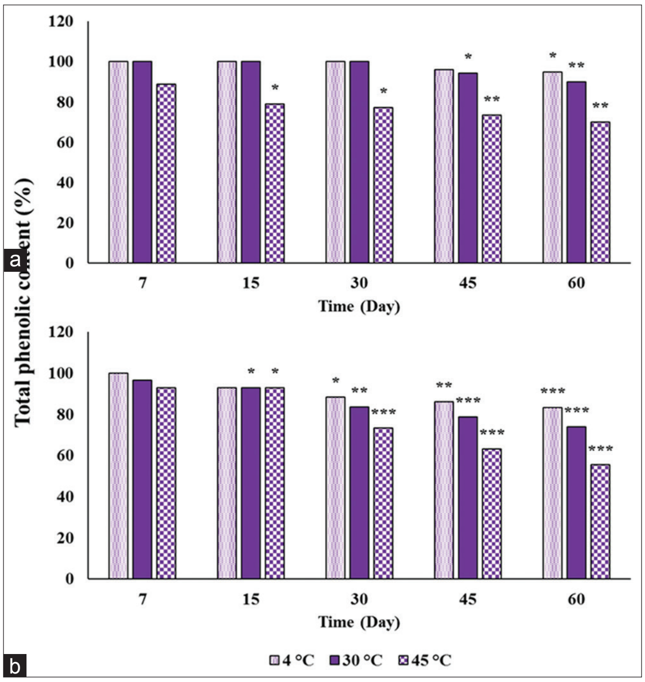

Fig. 3: The changes in the phenolic content of liquid soap (a) and solid soap (b) during storage at different temperature (4°C, 30°C, and 45°C).*, **, and * represent the significant variation with p>0.05, 0.01, and 0.001, respectively**

The stability study suggested that the storage temperature affects the active compound of the product. The storage at room temperature (30°C) also influences the phenolic content of the products, especially hard soap is more vulnerable to the temperature. However, the products retain at least 70% of its active principles, which suggested that the formulation was acceptable for further evaluation and marketing.

The heavy metal contamination in the cosmetic products was also assessed. Lead and barium were found in the mineral spray, shampoo, and soap products. The amount of lead and barium was <0.13 ppm and <0.24 ppm, respectively, which is an acceptable range of heavy metals in the cosmetics. Hence, all the products passed the heavy metal quality check (Table 6). The cosmetic products were analyzed for the microbiological safety and found that there was no culturable microbial content in the cosmetic samples (Table 7).

The results revealed that the cosmetic products are safe for human use and are free from heavy metal and microbial contamination. The cosmetic products are now intentionally made with natural compounds

Table 5: The stability of purple rice bran extract stored at 30°C

S. No.	Phenolic compounds	0 day	30 days	60 days
1	Protocatechuic acid	0.87±0.04	0.89±0.02	0.91±0.03
2	Caffeic acid	1.01±0.05	0.99±0.01	0.97±0.01
3	Syringic acid	0.19±0.01	0.19±0.00	0.19±0.01
4	*p*-coumaric acid	11.40±0.57	11.24±0.52	11.09±0.53

The values are represented as mg/g extract±SD. SD: Standard deviation

Table 6: The heavy metal contamination in the cosmetic formulas

Formulas	Heavy metal	Detected value	Result
Mineral spray	Lead*	<0.13 ppm	Pass
	Mercury*	Not detected	Pass
	Arsenic*	Not detected	Pass
	Barium*	0.24 ppm	Pass
Shampoo	Lead*	Not detected	Pass
	Mercury*	Not detected	Pass
	Arsenic*	Not detected	Pass
	Barium*	0.16 ppm	Pass
Liquid soap	Lead*	Not detected	Pass
	Mercury*	Not detected	Pass
	Arsenic*	Not detected	Pass
	Barium*	<0.13 ppm	Pass
Hard soap	Lead*	Not detected	Pass
	Mercury*	Not detected	Pass
	Arsenic*	Not detected	Pass
	Barium*	0.24 ppm	Pass

*The acceptable range of lead, mercury, arsenic, and barium are >20 ppm, >0.5 ppm, >5 ppm, and >500 ppm, respectively

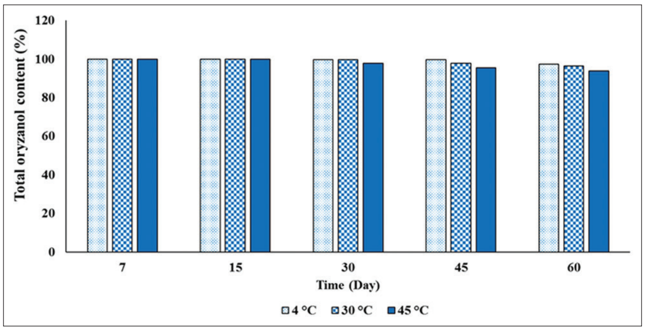

Fig. 4: The changes in the oryzanol content of the solid soap formulations during storage

Table 7: The microbiological analysis of cosmetic formulations

Formulations	Representative microbes	0–2 months
Mineral spray, shampoo, liquid soap, and solid soap	*Staphylococcus aureus*	Not detected
	Escherichia coli	Not detected
	Pseudomonas aeruginosa	Not detected
	Candida albicans	Not detected

as principal active ingredients. Some of the recent reports demand the use of fermented plant juice and alternative preservatives for the cosmetic preparations [26,27]. The rice and RB-derived compounds are used in cosmetic products, especially in moisturizer and skin care products [12,14].

Hanno *et al.* [15] reported the use of a rice-derived natural surfactant with emulsifying properties as a green cosmetic approach. *In vitro* cytotoxicity test and experiments in human volunteers proved that the rice panicle extract contains high levels of phenolic compounds, which provides the nourishment and acted as a skin antiaging agent [28]. Manosroi *et al.* reported that the RB bioactive compounds exhibit antiaging property while entrapped in niosomes. The results also proved that the formulation was a potent and novel skin care product [14].

CONCLUSION

The current study was a novel attempt to develop cosmetic products with PRE and RBO. Moreover, the present study also uses the fermented rice juice as a source of bioactive principle. We have already reported the use of fermented plant juice in cosmetic products [26]. The results of the current study suggested that the rice phytochemical-based cosmetic products are stable, free from heavy metals, and microbial contamination, and further fine-tuning of the formulation is needed to meet the market quality.

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