

## Chemical composition, physical properties, and sensory evaluation of an instant powder beverage containing melatonin prepared from vegetables

Wariya Hochin, Anuchita Moongngarm\*

Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University,  
Mahasarakham 44150, Thailand

\*Corresponding author: anuchitac@yahoo.co.th

### Abstract:

Melatonin is a naturally occurring compound *found* in plants, animals, and microorganism. It plays an important role in maintaining the body's *circadian rhythm in animals and it* has antioxidant properties. This study was carried out to develop an instant powder beverage using germinated red bean, lemon grass, and onion as sources of melatonin. The proportions of the components used in the beverage were determined using the Mixture Design Method, which generated instant beverages for 6 formulas including germinated red bean ( $X_1 = 30-50\%$ ), lemon grass ( $X_2 = 10-35\%$ ) and onion ( $X_3 = 15-42\%$ ). The effects of different proportions of raw materials on the appearance, color, texture and overall acceptability of the products were evaluated using a 9-Point hedonic scale. The results indicated that the proportions of germinated red bean: lemon grass: onion of 50:40:10 gained the highest score of acceptance. Six beverage powder formulas were also determined for physical properties and chemical composition. It was found that the beverage powder containing melatonin had suitable physicochemical properties, with respect to water absorption index (1.11 - 2.44 g / g), water soluble index (19.57 - 20.79%) and solubility (18.73 - 20.83 % residue). The study suggested that a melatonin-rich beverage powder could be produced as a functional food.

**Keywords:** Antioxidant, Functional food, Instant beverage, Biological rhythm, Body's *circadian rhythm*

## Introduction

Currently, consumer awareness about health has greatly increased. As a result, there is increasing consumption of healthy food products and a great demand of functional foods in the food market. A number of bioactive compounds from plants have been studied; however, there are only a few studies of melatonin. Melatonin (*N*-acetyl-5-methoxy tryptamine) [1] is a hormone produced by the pineal gland in animals. It regulates sleep and wakefulness [2]. Melatonin is also produced in plants to function as a defense against oxidative stress [3]. In animals, melatonin is involved in the synchronization of the circadian rhythms including sleep-wake timing, blood pressure regulation, seasonal reproduction, and other functions [4]. Many of its biological effects in animals are produced through the activation of melatonin receptors [5], its role as an antioxidant [6], with a particular role in the protection of nuclear and mitochondrial DNA [7]. Nowadays, more and more people of working-age want to rest for a limited time. Moreover, people who travel across time zones, as well as other consumers in general, need specific functional foods for better relaxation. Melatonin is a key bioactive compound to provide in functional foods for relaxation. From our previous study, we found that some vegetables contain high amounts of melatonin [1, 2, 8, 9]. Since vegetables are available throughout the year and inexpensive, they have the potential to use as a source of melatonin as a functional food for relaxation. However, consuming vegetables before sleeping may be inconvenient for consumers. Therefore, this study was conducted to develop an instant powder beverage as a functional drink product high in melatonin.

## Materials and methods

### *Raw materials*

Red beans (*Phaseolus vulgaris* Linn.), lemon grass (*Cymbopogon citratus* DC.Stapf.) and onions (*Allium cepa* Linn.) were purchased from local market in Mahasarakham province, Thailand. Red bean sprouts were prepared by germination of red bean seed for 3 days

### ***Preparation of instant powder beverage***

Germinated red beans, lemon grass, and onions were selected and used for developing an instant beverage. The vegetables were cleaned and cut into small sized pieces approximately 1x1 cm prior to boiling in hot water (80-90°C) until the texture of raw materials became soft. After that, the cooked vegetables were finely ground with water added (50% v/w) using a blender and filtered through cheese cloth. The samples were then reduced to dryness using freeze dryer. The dried samples were ground and used to develop the beverage. A total of 6 formulas of instant beverage were obtained using the mixture expert design program. These beverages were used for sensory evaluation, chemical composition analysis, and physical property determination. The sensory evaluation of this study was approved by the Human Ethics Committee, Maharakham University. The 9-Point Hedonic Scale technique was applied for sensory evaluation.

### ***Determination of melatonin***

#### ***Sample extraction for melatonin determination***

Vegetable samples were freeze dried and finely ground before use. Samples of 5.0 g were extracted with aqueous methanol (MeOH, 80%), 50 ml. The mixture was shaken in a shaking incubator for 30 min., then centrifuged for 16-22 hours at 2500 rpm. The supernatant was evaporated to dryness in a rotary evaporation machine, then re-suspended in MeOH (80%), 10 ml prior to analysis by HPLC-RP [10].

#### ***Melatonin determination***

The extract was filtered through a nylon filter syringe (0.45 µm). The HPLC system used to determine melatonin was a Shimadzu (Prominence HPLC, Shimadzu, Japan) with a fluorescent detector, 10A version and Series 20A, excitation wavelength at 290 nm and the emission wavelength at 330 nm, pumping system using an RF-20Axs. An analytical column used was RP-C18 column (4.6 x 250 mm, 5µm), (GL Science, Japan). The mobile phase consisted of solvent A (50 mM phosphate buffer at pH 7.2) and Solvent B (acetonitrile). The mobile phase was computer program-controlled for gradient elution as follows: (time, solvent B), (0.0 min, 0%), (5.00 min, 35%), (12.00 min, 40%), (20.00 min, 45 %), (25.00 min, 50%), (30.00 min, 0%), stopped at 40 min, with a flow rate of 1.0 mL/min. The injection volume was 20 µL. The concentration of melatonin in each sample was calculated using the area

under the graph obtained from the analysis comparing with a melatonin standard and the concentration was expressed as ng/g dry sample [10].

#### *Determination of chemical composition*

The proximate composition of the instant beverage samples was analyzed by the method of [11] including moisture content, ash, protein, fat, fiber, and carbohydrate.

#### *Determination of physical properties*

The physical properties measured in this study included water absorption index, water dissolution index, and the solubility (%).

### **Results and discussion**

#### *Melatonin content*

The melatonin content of the instant powder beverages and raw materials are indicated in Table 1.

It was found that all instant powder drinks were not significantly different in melatonin content which ranged from 15.18 to 19.18 ng/ g (dry sample). The level of melatonin found in this study was higher than those reported by Arnao et.al (2014) [12] and Padumanonda et.al (2014) [1]. The content of melatonin in plants can vary depending on several factors such as varieties [13], growing conditions, harvesting periods [14] and growth environment [15]. According to the report of a study of turnips [16], a melatonin dose of 0.5-5 µg could reduce jet lag. A melatonin dose of 0.1 µg per day has been applied to treat insomnia before a bedtime. This amount was enough for helping a person with insomnia to sleep if it provided a level of about 200pg in the blood [17]. If approximately 100 g of the instant beverage was taken, it could contribute a melatonin amount of about 1.6 µg. Therefore, this instant powder beverage is likely to be useful and has sufficient potential to warrant further development.

**Table 1.** Melatonin content in raw materials and instant powder beverages (ng/g dry sample)

<b>Formula</b>	<b>Germinated red bean (%)</b>	<b>Lemon Grass (%)</b>	<b>Onion (%)</b>	<b>Melatonin</b>
1	48	10	42	16.57±0.19
2	30	28	42	19.18±0.23
3	41.6	23.6	34.8	17.21±0.15
4	50	35	15	15.18±0.08
5	30	35	35	18.89±0.14
6	50	10	40	16.21±0.21
Raw material	100			7.30±0.26
Raw material		100		21.82±0.49
Raw material			100	25.92±0.86

#### *Sensory evaluation test of beverage powder with melatonin in 6 formulas*

The results of a sensory evaluation of the finished products of instant powder beverage, expressed as liking score (sensorial score), are shown in Table 2. It was found that all attributes including color, sweetness, saltiness, bitterness, smell and overall liking were significantly different in liking score ( $P < 0.05$ ), which the liking scores varied between 4.60 and 8.58. The formula 6 ingredient containing 50% red bean sprouts, 10% lemon grass and 40% onions obtained the best liking score in, sweetness, saltiness, bitterness, and smell, while liking scores of the powder beverage containing higher level of onion gained lower liking scores. This may due to the strong smell of onion itself.

#### *Analysis of Chemical compositions*

The results of chemical composition, including moisture content, ash, protein, fat, fiber, and carbohydrate of the instant powder beverage are shown in Table 3. The results indicated that the proportion of raw materials added to each formula significantly affected the moisture, ash, protein and fiber contents of the six powders ( $P < 0.05$ ), whereas the powders did not differ significantly in fat content ( $P > 0.05$ ). The moisture content, ash, fat, fiber, carbohydrate, and protein of the products ranged from 6.09 to 6.91%, 4.10 to 4.77%, 0.08 to 0.13%, 38.15 to 41.57%, 15.09 to 20.89% and 28.086-30.112%, respectively. The red bean is high in protein and it was added in high proportion in each formula resulting in the high

protein content in some of the beverages. Fiber content was high in lemon grass; as a result, this ingredient contributed high fiber content to the relevant beverages.

**Table 2.** Liking scores of instant powder beverages obtained using 9-point hedonic scale for evaluation

Formula	Germinated red bean (%)	Lemon Glass (%)	Onion (%)	Color	Sweetness	Bitterness	Saltiness	Odor	Overall liking
1	48	10	42	6.47±0.82 <sup>b</sup>	6.87±0.63 <sup>b</sup>	6.63±0.67 <sup>b</sup>	6.73±0.74 <sup>b</sup>	6.73±0.74 <sup>b</sup>	7.06±0.43 <sup>b</sup>
2	30	28	42	4.17±0.59 <sup>c</sup>	5.13±0.82 <sup>d</sup>	5.43±0.63 <sup>c</sup>	4.33±0.76 <sup>e</sup>	5.40±0.56 <sup>e</sup>	4.60±0.61 <sup>d</sup>
3	41.6	23.6	34.8	6.80±0.85 <sup>b</sup>	5.93±0.69 <sup>c</sup>	6.10±0.88 <sup>b</sup>	6.03±0.72 <sup>c</sup>	6.13±0.57 <sup>c</sup>	6.93±0.61 <sup>c</sup>
4	50	35	15	8.03±1.00 <sup>a</sup>	8.00±0.83 <sup>a</sup>	8.07±0.83 <sup>a</sup>	8.03±0.56 <sup>b</sup>	8.03±0.56 <sup>b</sup>	8.23±0.79 <sup>a</sup>
5	30	35	35	6.13±0.97 <sup>b</sup>	5.77±0.82 <sup>c</sup>	5.10±0.76 <sup>c</sup>	5.33±0.66 <sup>d</sup>	6.03±0.56 <sup>d</sup>	5.50±0.72 <sup>c</sup>
6	50	10	40	8.06±0.81 <sup>a</sup>	8.23±0.80 <sup>a</sup>	8.06±0.81 <sup>a</sup>	8.13±0.85 <sup>a</sup>	8.19±0.75 <sup>a</sup>	8.58±0.70 <sup>a</sup>

Means within a column with different superscript letters (a, b, c, ...) are different ( $p < 0.05$ )

**Table 3.** Chemical composition of instant powder beverages

Formula	Germinated red bean (%)	Lemon Glass (%)	Onion (%)	Moisture content (%)	Ash (%)	Protein (%)	Fat <sup>ns</sup> (%)	Fiber (%)	Carbohydrate (%)
1	48	10	42	6.31±0.07 <sup>c</sup>	4.22±0.05 <sup>d</sup>	18.36±0.09 <sup>b</sup>	0.08±0.04	38.96±0.54 <sup>bc</sup>	28±1.15 <sup>bc</sup>
2	30	28	42	6.09±0.07 <sup>d</sup>	4.10±0.01 <sup>e</sup>	15.09±0.04 <sup>c</sup>	0.12±0.07	38.15±1.50 <sup>bc</sup>	28±0.96 <sup>bc</sup>
3	41.6	23.6	34.8	6.30±0.06 <sup>c</sup>	4.19±0.06 <sup>d</sup>	18.42±0.09 <sup>b</sup>	0.13±0.03	38.05±1.09 <sup>c</sup>	28±1.08 <sup>bc</sup>
4	50	35	15	6.52±0.17 <sup>b</sup>	4.73±0.06 <sup>a</sup>	20.77±0.10 <sup>a</sup>	0.10±0.03	40.44±1.42 <sup>b</sup>	30±1.12 <sup>b</sup>
5	30	35	35	6.91±0.04 <sup>a</sup>	4.77±0.06 <sup>a</sup>	15.85±0.24 <sup>a</sup>	0.11±0.03	38.64±1.61 <sup>bc</sup>	28±0.97 <sup>bc</sup>
6	50	10	40	6.55±0.03 <sup>b</sup>	4.28±0.14 <sup>c</sup>	20.89±0.09 <sup>a</sup>	0.13±0.03	41.57±0.66 <sup>a</sup>	30±0.16 <sup>a</sup>

Means within a column with different superscript letters (a, b, c, ...) are different ( $p < 0.05$ )

**Table 4.** Physical properties of instant powder beverages

Formula	Germinated red bean (%)	Lemon Glass (%)	Onion (%)	Water absorption index (g/g)	Water soluble index (%)	Water soluble ability (%residue)
1	48	10	42	2.44±0.07 <sup>a</sup>	20.79±0.10 <sup>a</sup>	18.73±0.41 <sup>c</sup>
2	30	28	42	1.11±0.06 <sup>d</sup>	19.57±0.06 <sup>b</sup>	20.83±0.10 <sup>a</sup>
3	41.6	23.6	34.8	1.81±0.03 <sup>c</sup>	19.92±0.07 <sup>b</sup>	19.73±0.09 <sup>b</sup>
4	50	35	15	1.76±0.07 <sup>c</sup>	19.60±0.17 <sup>b</sup>	20.49±0.31 <sup>a</sup>
5	30	35	35	1.83±0.06 <sup>c</sup>	19.70±0.34 <sup>b</sup>	19.57±0.40 <sup>b</sup>
6	50	10	40	2.26±0.05 <sup>b</sup>	20.58±0.50 <sup>a</sup>	19.53±0.45 <sup>b</sup>

Means within a column with different superscript letters (a, b, c, ...) are different ( $p < 0.05$ )

### ***Analysis of Physical compositions***

The water absorption index (WAI), solubility index (WSI) and solubility (WSA) of instant beverage powder are presented in Table 4. The results indicated that the WAI, WSI, and WSA of products were significantly affected by the proportions of raw materials ( $P < 0.05$ ). The WAI, WSI, and WSA varied from 1.11 to 2.44 g/g, 19.57 to 20.79% and 18.73 to 20.83%, respectively. The WAI of the food product depends on the amounts of soluble dietary fiber [18], protein, and fat. If products contain high levels of fiber and protein but low fat content, a high WAI is expected [19]. Formulas 1 and 6 contain higher proportions of red bean than others (*except Formula 4!!*); as a result in higher level of protein, therefore, the WAI was higher than other formulas.

### **Conclusions**

This study has demonstrated the feasibility of developing an instant powder beverage as a functional drink product containing melatonin. The six trial products obtained were acceptable by panelists with generally high liking scores. The products all contained melatonin; however, its concentration in the products still need to be increased to ensure effectiveness. the amount of melatonin which this may be achieved by extracting the melatonin from the raw materials prior to added it back to the finished product.

### **Acknowledgements**

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