The Optimal Formula of Longan Energy Gel Drink Containing of High Bioactive Compounds and Antioxidant Activities

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Abstract— **Energy gel drink tends to be more popular as functional drink. Bioactive compounds and antioxidant activities were interested to study in this type of drink. The objective of this study was to find out the optimal formula for main ingredient, minor ingredient and gelling agent of longan energy gel drink. It was found that the optimal formula for main ingredient consisted of 69.5% longan juice, 30% longan honey and 0.5% citric acid which had 25 °Brix TSS and 0.5% TA (as citric acid). The mixture of main ingredient was added with minor ingredient which consisted of 0.54% branch chain amino acid (BCAA), 0.11% vitamin B complex and 0.03% commercial caffeine powder. Carboxymethyl cellulose (CMC) was selected to be added at 0.7% for gelling agent since no syneresis and the high acceptance sensory scores. Longan energy gel drink mixture was filled (150 g), sealed in retortable pouch and boiled in water (95-97 °C) for 5 min. One serving size (150 g) of this product had 165 kcal. In addition, it also had high bioactive compounds such as 0.401±0.009 mg GAE/ml of total phenolics, 0.013±0.002 mg/ml of gallic acid and 0.005±0.001 mg/ml of ellagic acid. These bioactive compounds could also provide high antioxidant activities. This longan energy gel drink had high potential to produce at commercially scale.**

Index Terms—**longan, energy gel drink, gelling agent, bioactive compound, antioxidant activity**

I. Introduction

Functional drinks can provide some health benefit compounds which are vitamins, minerals and polyphenols [1]. One of the popular functional drinks is energy drink. Thai market segmentation of energy drinks in 2003 was 62.40% of the functional beverage category [2]. Energy drink market has been growing continuously. In USA, evaluated sale of energy drink industry increase of 60% from 2008 to 2012. Sales in 2012 were 12.5 billion USD [3].

The energy drink can provide energy immediately, alertness and good performance to the consumer body. Main energy sources are sugars and their derivatives [4].

The northern part of Thailand is the main area for fruit production. In 2011, longan fruits (*Dimocarpus longan*, Lour.) were produced 777,099 tons [5]. Most of longan fruits were produced as whole fruit dried longans which were almost exported to China. It was reported that drying process could enhanced the contents of gallic acid, ellagic acid and phenolic compounds [6]. These chemicals were health benefit compounds according to their antioxidant activities. Many researchers reported that gallic acid and ellagic acid had efficient to be anti-inflammatory, antimicrobial and anticancer agents [7], [8], [9]. During longan flower blooming, longan trees can also provide longan honey for honey industries. Longan honey has glucose and fructose as energy sources which can be rapidly absorbed to blood stream after consumption [10]. This research was interested to use substandard fruit size of whole fruit dried longan to prepare longan juice. Longan juice was mixed with longan honey and then acidified with citric acid.

For minor ingredients in energy drink usually consists of caffeine, taurine, branch chain amino acid and vitamins [11]. Many researchers reported that caffeine consumption improved concentration, mood and exercise performance [12], [13]. There are some vitamins in vitamin B complex such as vitamin B3 which helps to improve energy metabolisms as coenzyme and vitamin B12 which supports in nerve functions [11]. Branched-chain amino acid (BCAA) was also interested to use as minor ingredient. BCAA could support muscle building and exercised muscle damage reduction [14], [15].

Energy drinks in Thailand are mostly in liquid form which was packed in glass bottles. Glass containers are heavy, easy to break, not convenient to consumption during sport activities. This research would like to develop energy drink to be gel form that was filled in flexible plastic bags which are convenient for consumption and transportation.

II. Materials and Methods

A. Materials

Substandard fruit size of whole fruit dried longan was purchased from dried longan factory in Chiang Mai, Thailand. Other main ingredients were longan honey (Supha bee Co., Ltd., Chaing mai, Thailand) and commercial citric acid. The minor ingredients included of caffeine powder (DKSH Management Ltd., Bangkok,

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Thailand), vitamin B complex (Adinop Co., Ltd., Bangkok, Thailand) and branched-chain amino acid (BCAA) (VX Nutrition Co., Ltd., USA). The gelling agents consisted of carboxymethyl cellulose (CMC), konjac glucomannan, xanthan gum and pectin which were purchased from Yok Interade Co., Ltd., Chiang Mai, Thailand. Arenga gum was prepared from sweet palm seed followed by Jomduang S., and Syahirah N. F. Z. A. (2018) [16]. Commercial gel drink purchased from a local convenient store.

B. Longan Juice Preparation

Whole fruit dried longan were peeled, washed and boiled in water at the ratio of 1:10 (w/w) for 30 min. The longan juice solution was derived from hydrolic pressing, filter cloth filtering and water adding for adjusting to the previous ratio.

C. Energy Gel Drink Preparation

Longan juice solution was mixed with different amount of longan honey to provide the certain total soluble solid (TSS) of the mixture. After mixing, the solution was acidified with citric acid to provide the certain total acidity (TA). After mixing, the solution was added with minor ingredients (BCAA and vitamin B complex). Caffeine powder was added at 50 mg perserving size according to Thai Food and Drug Administration regulation. Prepared solution was preheated before mixing with a gelling agent which was CMC, konjac glucomannan, xanthan gum, pectin and arenga gum. Energy gel mixture was filled (150 g/bag), sealed in retortable pouch and boiled in hot water (95-97 ˚C) for 15 min.

D. Determination of Physical Properties

Color values: CIE L* a* b* were measured using colorimeter (model Color Quest XE, Hunter Lab).

Viscosity: Brookfield viscometer (DV-II+Pro, USA) was used to measure using probe number 18, 1 rpm at 25 ˚C

Firmness: Texture analyzer (TA.XT. Plus, Stable Micro systems, UK) was used to measure the peak force (g) using back extrusion technique [17]. Cylinder probe (A/BE-d35) was penetrated into the sample solution at 30 mm depth with 5 g load.

Syneresis: Syneresis determination was measured using centrifuge followed Ramírez-Sucre *et al*. 2013 method [18].

E. Determination of Chemical Properties

Total solubility solid (TSS): TSS was measured using a hand refractometer (Atago N-1E, Japan) [19].

pH: pH was measured using pH meter (Cyber: Model scan-510, Singapore) [19].

Total acidity (TA): TA was determined using titration method with 0.1 N NaOH. The result was reported %TA as citric acid [19].

Moisture, protein, carbohydrate, fat and ash were determined using AOAC method [19].

Energy: Total energy per serving size was calculated from its energy of protein, carbohydrate and fat content. [20]

F. Determination of Gallic Acid and Ellagic Acid

Gallic acid and ellagic acid were measured using HPLC (Agilent Technologies 1260 Infinity, USA) followed Chaikham and Apichartsrangkoon, (2012) method [21].

G. Determination of Total Phenolic Compound

Sample extraction was adapted from Jalgaonkar et al. (2018) [21]. The extracted sample solution (0.25 ml) was mixed with distilled water (3 ml), Folin–Ciocalteu reagent (0.25 ml) and 7% Na_2CO_3 solution (2.5 ml). And then kept in the dark room for 30 min. Solution absorbance was determined at 760 nm compared to gallic acid standard solution. Total phenolic compound was reported in mg as gallic acid equivalent per ml sample (mg GAE/ml) [22].

H. Determination of Antioxidant Activities

Sample extraction was adapted from Jalgaonkar *et al*. (2018) [23].

DPPH assay: The extracted sample solution (3 ml) was mixed with 0.1 mM (2,2-diphenyl-1-picrylhydrazyl) DPPH (1 ml). And then kept in the dark room for 30 min. Solution absorbance was detected at 517 nm. DPPH assay was reported in mg as trolox per ml [24].

ABTS assay: The extracted sample solution (100 µl) was mixed with 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) and ABTS solution (1.9 ml) [25]. And then kept in the dark room for 6 min. Solution absorbance was detected at 734 nm compared to trolox solution. ABTS assay was reported in mg trolox per ml.

FRAP assay: FRAP (Ferric Reducing Antioxidant Power) working solution was mixed with extracted sample solution (50 µl) [23]. And then kept in the dark room for 4 min. Solution absorbance was detected at 593 nm compared to ferrous sulphate solution. FRAP assay was reported in mg as Fe (II)/ml.

I. Determination of Microbiological Load

Analysis of total plate count, coliform bacteria, *Escherichia coli*, yeast and mold in finished longan energy gel drink was followed the method of the Bacteriological Analytical Manual (2001) [26].

J. Sensory Evaluation

Sensory evaluation was performed using 50 panelists and 1-9 point hedonic scales of appearance, color, odor, texture, flavor and overall acceptance of samples were evaluated.

III. Results and Discussions

A. Optimal Formulation of Main Ingredient

In this study, response surface methodology (RSM) with 2 factors of central composite design (CCD) was used for formula optimization. Two factors were total soluble solid (TSS) (18-26 ˚Brix) and citric acid (0.2-0.7%). It was shown that formula 9 had the highest palatability scores (6.31±1.86) (Table I.). After RSM plotting, it was shown that the regression equation for palatability scores had 0.88 of coefficients of determination (R^2) (Fig. 1). This equation could use to predict the optimal formula which had 25.41°Brix and 0.5% citric acid. It could provide 6.38 palatability scores. From this formula, the main ingredient consisted of 69.5% longan juice, 30% longan honey and 0.5% citric acid.

TABLE I. PALATABILITY SCORES FROM DIFFERENT MAIN INGREDIENT FORMULA OF LONGAN ENERGY GEL DRINK

Formula	Main ingredients composition		Palatability evaluation (1-9)
	TSS (°Brix)	Citric acid content (%)	
1	18.00	0.20	6.04^{a} ±1.87
2	26.00	0.20	6.00^{a} ±1.93
3	18.00	0.70	4.35^{cd} ±2.06
4	26.00	0.70	5.73^{ab} ±2.02
5	16.34	0.45	4.82^{cd} ±1.90
6	27.66	0.45	6.04^{a} ±1.90
7	22.00	0.10	5.02^{bc} ±1.99
8	22.00	0.80	4.06^{d} ±1.80
9	22.00	0.45	6.31^{a} ±1.86

$^{a\text{-}d}$ means in the same column with different superscripts are significantly different (P≤0.05)

Palatability scores = 0.77135◦Brix + 1.31318acidity – 0.018984◦Brix2 – 11.98000acidity2 + 0.35500 ◦Brix × acidity – 3.15084 R^2=0.88

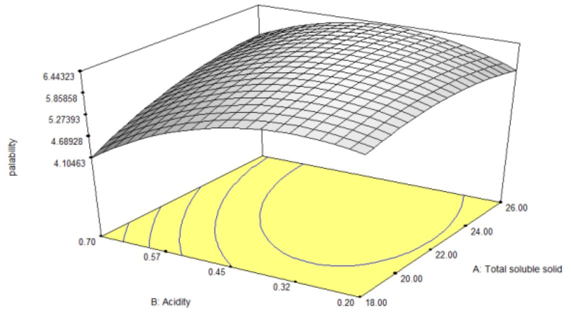

Figure 1. Response surface for palatability of main ingredient in longan energy gel drink

B. Optimal Formulation of Minor Ingredients

This experiment was designed using RSM with 2 factors of CCD. Two factors were 0-6.5 g BCAA and 0-0.15 g vitamin B complex per serving size (150 g). BCAA addition increased total acidity which was related to Hone *et al.* (2018) [27] study (Table II). It was shown that vitamin B complex (yellow powder) addition effected to color value, the higher amount of vitamin B complex addition, the higher of yellow value (b*). From the RSM plotting, it was shown that the regression equation of flavor scores had 0.99 of R^2 (Fig. 2). According to this equation, the optimal composition consisted of 0 g BCAA and 0.17 g vitamin B complex per serving size which could predict about 6.76 of flavor evaluation scores. In this study, 0.17 g per serving size of vitamin B complex addition was selected. Consideration to BCAA addition which was added followed the supplier recommendation (6.5 g/serving size), it was found that BCAA decreased flavor evaluation scores. That amount was too high for this product addition. In the next study, the maximum amount of BCAA addition was only half amount of supplier recommendation (3.25 g/serving size). From longan energy gel drink products which were prepared from fix amount of vitamin B complex (0.17 g/serving size) and various amount of BCAA addition, they had a little bit different of color values and titratable acidity. It was found that BCAA addition at 0.81 g/serving size had the similar flavor evaluation scores to non-addition, not statistical different (p>0.05) (Table III). From the previous study of Wang, Soyama, and Luo (2016) [28] about novel functional drink, BCAA addition was selected to add at only 500 mg/100 ml or 0.75 g/150 ml. Consideration to the main ingredient, the minor ingredients were added up at 0.54% BCAA, 0.11% vitamin B complex and 0.03% caffeine.

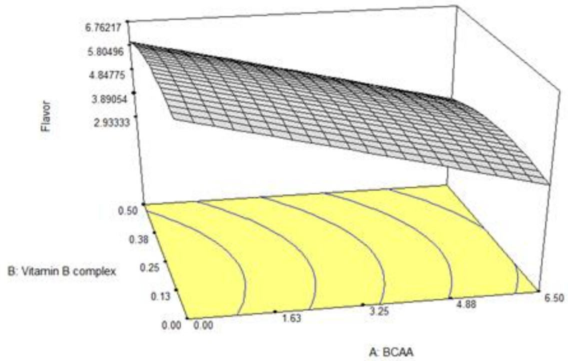

Figure 2. Response surface for flavor of minor ingredient in longan energy gel drink

TABLE II. SOME QUALITIES FROM DIFFERENT MINOR INGREDIENTS FORMULA OF LONGAN ENERGY GEL DRINK

Formula	Minor ingredients composition (g/serving size)[1]		Color values			Titratable acidity (% as citric acid)	pH	Flavor evaluation (1-9)
	BCAA	Vitamin B complex	L*	a*	b*			
1	0	0	27.94^{abc} ±2.22	-0.57^{a} ±1.36	6.47^{d} ±2.48	0.52^{d} ±0.01	3.24^{h} ±0.01	6.51^{ab} ± 2.09
2	6.5	0	27.02^{d} ±0.04	0.19^{a} ±0.09	8.26^{c} ±0.07	0.66^{a} ±0.01	3.79^{b} ±0.01	3.46^{f} ± 2.06
3	0	0.5	28.54^{abc} ±0.07	-2.85^{bcd} ±0.06	14.03^{ab} ±0.05	0.53^{d} ±0.01	3.37^{f} ±0.01	5.92^{b} ± 1.74
4	6.5	0.5	27.90^{abc} ±0.05	-2.30^{bc} ±0.03	13.03^{ab} ±0.09	0.62^{b} ±0.03	3.83^{a} ±0.01	3.03^{f} ± 2.02
5	0	0.25	29.07^{ab} ±0.05	-2.87^{bc} ±0.08	12.70^{b} ±0.11	0.51^{d} ±0.01	3.34^{g} ±0.01	6.82^{a} ± 1.54
6	6.5	0.25	28.35^{abcd} ±0.03	-2.24^{b} ±0.06	12.60^{b} ±0.05	0.68^{a} ±0.01	3.78^{b} ±0.01	3.38^{f} ± 2.03
7	3.25	0	27.48^{cd} ±0.04	-0.18^{a} ±0.09	7.81^{cd} ±0.12	0.60^{b} ±0.01	3.63^{e} ±0.01	4.90^{cd} ± 2.04
8	3.25	0.5	29.24^{ab} ±0.13	-3.45^{d} ±0.06	14.24^{a} ±0.06	0.60^{b} ±0.02	3.67^{c} ±0.01	4.44^{d} ± 2.06
9	3.25	0.25	29.39^{a} ±0.10	-3.11^{cd} ±0.06	12.52^{b} ±0.07	0.56^{c} ±0.01	3.64d ±0.01	5.28^{c} ± 1.96

[1] serving size = 150 g

$^{a\text{-}g}$ means in the same column with different superscripts are significantly different (P≤0.05)

C. Selection of Gelling Agent

From the viscosity determination of a commercial energy gel product, it had 695.24±180.50 cps which was used as the viscosity guideline. When the viscosity of different type of gelling agent concentrations were studied at the similar viscosity guideline from CMC, konjac glucomannan, xanthan gum, pectin and arenga gum, the concentration was 0.7, 0.4, 0.2, 2.5 and 2.2 % ,respectively. After longan energy gel preparation with different type of gelling agent addition at the resulted concentration, their viscosity were similar, non-statistical different (p>0.05) (in the range of 607.40 – 676.97 cps) (Table IV). Their Color value (L*, a*, b*), acidity, pH and firmness were a little bit different. In this study, the main criterion for gelling agent selection was 0 % syneresis. Syneresis properties effected on the first sight consumer acceptance of this type product. It was founded that only 0.7% CMC, 0.2% xanthan gum and 2.5% pectin addition had no syneresis. After longan energy gel preparation using different selected gelling agents, It was found that only the sensory evaluation scores of odor were similar, the other sensory characteristics were different (Table V). Adding 0.7% CMC could provide the highest sensory evaluation scores in appearance, color, texture and overall acceptance. It was indicated that 0.7% CMC was suitable to use as gelling agent in this product. This result was similar to Somogyi, L. P. (1996) [29] study which 0.5% CMC was used at thickening agent in fruit juice product.

TABLE III. SOME QUALITIES FROM DIFFERENT AMOUNT OF BCAA IN LONGAN ENERGY GEL DRINK

Qualities	BCAA content (g/serving size)				
	0	0.81	1.625	2.43	3.25
Color values					
L*	27.38^{c} ±0.12	28.05^{b} ±0.52	28.90^{a} ±0.11	28.94^{a} ±0.21	28.70^{a} ±0.24
a*	-0.44^{a} ±0.13	-0.79^{b} ±0.11	-1.06^{c} ±0.04	-1.03^{c} ±0.03	-1.02^{c} ±0.06
b*	7.77^{a} ±0.20	7.42^{ab} ±0.38	6.87^{c} ±0.31	6.92^{bc} ±0.21	6.90^{bc} ±0.25
Titratable acidity^{ns} (% as citric acid)	0.61±0.03	0.61±0.01	0.63±0.02	0.64±0.01	0.64±0.01
pH	3.05^{e}±0.01	3.21^{d}±0.01	3.30^{c}±0.01	3.42^{b}±0.01	3.45^{a}±0.01
Flavor evaluation (1-9)	7.19^{a} ±1.52	6.52^{ab} ±1.42	6.15^{b} ±1.60	5.07^{c} ±1.68	4.78^{c} ±1.76

ns = non-significant

$^{a-e}$ means in the same row with different superscripts are significantly different (P≤0.05)

Flavor scores = 6.55333 - 0.48103BCAA + 2.45333vitamin B complex – 1.89349E-003BCAA^2 – 7.20000vitamin B complex^2 + 0.049231BCAA×vitmain B complex R^2=0.99

TABLE IV. QUALITIES OF PHYSICAL AND CHEMICAL OF DIFFERENT TYPE AND CONCENTRATE GELLING AGENTS IN LONGAN ENERGY GEL DRINK

Qualities	Type and concentration of gelling agent				
	CMC (0.7%)	Arenga gum (2.2%)	Konjac glucomannan (0.4%)	Xanthan gum (0.2%)	Pectin (2.5%)
Viscosity^{ns} (cps)	641.90±62.05	625.40±109.50	612.95±125.26	676.97±118.52	607.40±80.85
Color values					
L*	31.50^{ab}±0.41	31.10^{b}±1.66	31.20^{ab}±1.27	30.93^{b}±1.18	31.95^{a}±0.80
a*	0.31^{a}±0.12	-0.50^{c}±0.16	-0.25^{b}±0.03	-0.34^{bc}±0.26	-0.42^{bc}±0.42
b*	3.79^{b}±0.19	3.14^{b}±0.69	3.82^{b}±0.09	3.81^{b}±0.04	4.57^{a}±0.20
Titratable acidity (% as citric acid)	0.61^{b}±0.01	0.57^{c}±0.02	0.61^{b}±0.05	0.61^{b}±0.03	0.74^{a}±0.02
pH	3.66^{a}±0.03	3.41^{b}±0.01	3.44^{b}±0.02	3.43^{b}±0.02	3.32^{c}±0.08
Firmness (g)	18.70^{ab}±1.47	19.85^{a}±1.16	17.73^{b}±0.42	19.48^{ab}±0.42	19.74^{ab}±0.67
Syneresis (%)	0	56.34±0.13	87.07±4.23	0	0

0 = no syneresis , ns = non-significant

$^{a-c}$ means in the same row with different superscripts are significantly different (P≤0.05)

D. Optimal Time of Pasteurization

Longan energy gel drink was packed (150 g) in retort pouch, sealed and boiled in hot water at 95-97 °C with different pasteurization times (at 5, 10 and 15 min). From the microbiological determination, all pasteurization time had the similar results; < 0.3 MPN/ml coliform bacteria, < 0.3 MPN/ml *Escherichia coli*, < 1 CFU/ml total plate count and < 1 CFU/ml Yeasts & Molds of 5, 10 and 15 min. These qualities were in the food safety values according to Thai Food and Drug Administration regulation. It was indicated that boiling at 95-97 °C for 5 min was suitable for longan energy gel drink pasteurization.

E. Bioactive Compound Contents, Antioxidant Activities and Energy of Finised Longan Energy Gel Drink

Longan energy gel drink had similar amount of gallic acid and ellagic acid to longan juice (Table VI). Longan honey addition helped to increase total phenolic compounds content in finished product. The finished product of longan energy gel drink had high antioxidant activities similar to longan juice and longan honey. Longan energy gel drink contained 162.97 kcal per serving size of energy which was similar to some commercial energy normal drink [11]. In this longan energy gel drink, main energy sources were provided from original longan juice and longan honey which had high content of fructose, glucose and sucrose. These sugars could be metabolized to energy easily than polysaccharide [30]. Therefore this gel drink could provide immediately energy after consumption. In some commercial energy gel drinks which were claimed about 300kcal per serving size (100g), about 50% of energy source was maltodextrin which was metabolized slowly. In addition, this novel product could also claim high phenolic compounds and bioactive activities. This studied product had high potential to scale up at commercial product level.

TABLE V. SENSORY EVALUATION OF LONGAN ENERGY GEL DRINK AT DIFFERENT TYPE OF GELLING AGENTS

Sensory evaluation	Formulations		
	CMC (0.7%)	Pectin (2.5%)	Xanthan gum (0.2%)
Appearance	$7.58^{a}\pm0.90$	$7.68^{a}\pm1.00$	$5.40^{b}\pm1.55$
Color	$7.50^{a}\pm0.85$	$7.25^{a}\pm1.13$	$6.40^{b}\pm1.15$
Odor^{ns}	7.02 ± 1.27	6.82 ± 1.15	6.95 ± 1.20
Texture	$6.75^{a}\pm1.58$	$6.45^{ab}\pm1.52$	$6.28^{b}\pm1.30$
Overall acceptance	$7.02^{a}\pm1.14$	$6.58^{b}\pm1.08$	$6.23^{b}\pm1.29$

ns = non-significant
$^{a-b}$ means in the same row with different superscripts are significantly different (P≤0.05)

TABLE VI. BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES COMPARED TO LONGAN ENERGY GEL DRINK, LONGAN JUICE AND LONGAN HONEY

Bioactive compounds and antioxidant activities	Longan energy gel drink	Longan juice	Longan honey
Gallic acid (mg/ml)	0.013±0.002	0.012±0.001	-
Ellagic acid (mg/ml)	0.005±0.001	0.006±0.001	-
Total phenolic compounds (mg GAE/ml)	0.401±0.009	0.294±0.029	0.516±0.012
DPPH assay (mg trolox/ml)	0.150±0.072	0.179±0.014	0.031±0.042
FRAP assay (mg Fe(II)/ml)	2.110±0.015	2.096±0.234	1.973±0.295
ABTS assay (mg trolox/ml)	0.227±0.014	0.266±0.029	0.286±0.051
Energy (kcal/100 g)	108.65	10.37	333.81
Energy (kcal/servingsize)	162.97	-	-

IV. CONCLUSION

The main ingredient of longan energy gel drink consisted of 69.5% longan juice, 30% longan honey and 0.5% citric acid which had 25 °brix of TSS and 0.5% TA as citric acid. The minor ingredient, it was added up and mixed with 0.54% BCAA, 0.11% vitamin B complex and 0.03% caffeine. CMC was selected to be the optimal of gelling agent which was added at 0.7% CMC. The mixture of longan energy gel drink was filled and sealed in 150 g bag size and then heat up to hot water (95-97 °C) for 5 min. One serving size (150 g) contained of 163 kcal and high bioactive compounds such as 0.013±0.002 mg/ml of gallic acid and 0.005±0.001 mg/ml of ellagic acid and antioxidant activities.

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