

Integrated solvent-free extraction and encapsulation of lutein from marigold petals and its application

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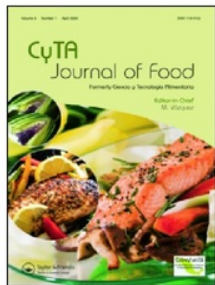
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4 Integrated solvent-free extraction and encapsulation of lutein from marigold petals and its application

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ABSTRACT

Vivid orange petals of marigold have been studied well as the richest source of lutein. Although it is very promising to be explored as functional food ingredients, there were only few studies that described extraction methods which meet food grade requirements. Here, lutein was extracted from marigold petals using vegetable oil, subsequently encapsulated and prepared ¹² lyophilized powder, and incorporated into milk-tea beverage. An organoleptic assay was conducted in order to evaluate the effect of lutein addition into milk-tea beverage as well as the consumers' preference. The results showed that the extraction capacity of vegetable oil was 1.9 times higher than that of acetone, being comparable to that of hexane. Interestingly, the encapsulated lutein was favorably incorporated into milk-tea, and its flavor was liked very much by the panellists.

Extracción integrada sin solventes y encapsulación de luteína procedente de pétalos de caléndula y su aplicación

RESUMEN

Existen muchos estudios sobre los pétalos de color naranja intenso de la caléndula [botón de oro, mercadela o maravilla], debido a que constituyen la fuente más rica de luteína. Aunque las investigaciones que analizan los ingredientes de los pétalos en cuanto alimento funcional son prometedoras, existen pocos estudios que proporcionen descripciones de métodos de extracción que cumplan con los requisitos necesarios para obtener el grado alimenticio. Para la presente investigación se extrajo luteína de pétalos de caléndula utilizando aceite vegetal; luego, ésta fue encapsulada y preparada como polvo liofilizado e incorporado en bebidas de té con leche. Posteriormente, se realizó una prueba organoléptica destinada a evaluar el efecto de adicionar luteína en bebidas de té con leche y medir su preferencia entre los consumidores. Los resultados al respecto dan cuenta de que la capacidad extractiva del aceite vegetal fue 1.9 veces más elevada que la de la acetona y comparable con la del hexano. Curiosamente, la luteína encapsulada fue incorporada de manera favorable en bebidas de té con leche y su sabor resultó muy agradable a los panelistas.

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Beverage; lutein; marigold; organoleptic; organic solvent-free extraction



PALABRAS CLAVE



Bebida; luteína; caléndula; organoléptico; extracción orgánica sin solventes

1. Introduction

Marigold (*Tagetes erecta* L., local name: gumitir) farm in Bali has become one of the most iconic flower gardens in Indonesia. These flowers, having yellow to orange petals, are widely cultivated for supporting agro-tourism in Bali, meeting the need of local community for religious ceremonies, and some of the rest are used as veterinar ²² feed. Interestingly, the marigold petals are the rich source of carotenoids, such as lutein, β -carotene, lycopene, and zeaxanthin (Bolanos, Cruz, Islas, Alvarez, & Ramiro, 2005; Siriamornpun, Kaisoon, & Meeso, 2012). In particular, the dominance of lutein brings out major interest. Recent investigations have clearly verified that lutein belonged to principal carotenoids in ²⁸ human brain and ocular tissues (Bernstein et al., 2001; Johnson et al., 2016). It plays an important role in neural development, better cognitive ²¹ performance, protection against macular disease, as well as may have some biological effects including antioxidant, anti-inflammation, and structural actions (Johnson, 2014). Higher plasma lutein was related ¹⁷ to improvisation in visual function of the community with long-term

computer display light exposure (Ma, Xiao, Zhi, & Xian, 2009), delayed cellular senescence, and age-related diseases (Sen et al., 2014), as well as better recall and verbal fluency in elderly (Johnson, 2012). For such great benefits, marigold flower could be explored as an inexpensive indigenous ingredient for the development of functional food product.

A number of studies have dealt with lutein extraction from marigold petals. Despite the fact that exhaustive extraction can only be achieved by organic solvents, the consumers concern and safety issues may ³⁰ lead to the construction of solvent-free extraction technique. Gao, Nagy, Liu, Simandi, and ⁷ Wang (2009) and Gao et al. (2010) have worked on supercritical CO₂ extraction of lutein esters from Chinese marigold which were combined with either vegetable oil (as co-solvent) or ultra-sonication, being comparable to the study carried out by Palumpitag, Prasitchoke, Goto, and Artiwan in Thailand (2011). The use of edible oil offers several important green impacts such as the reduction of organic solvents originated from non-renewable resource, consuming less energy, increasing the product quality, and producing less chemical waste to the environment (Chemat et al., 2017; Yara-Var

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ón et al., 2017). To the best of our knowledge, there are only very limited studies that emphasize the functionality of lutein from marigold varieties grown in Bali, Indonesia, except the identification and stability study conducted by Aida (2015) in collaboration with our research group.

Furthermore, considering the extensive and exponential growth of functional food development, we decided to incorporate marigold's lutein in ordinary food product, instead of dietary supplement. In fact, consumers of dietary supplement are mostly concerned with the uncertain effective dosing, side-effects, and potential interaction with certain drugs (Halsted, 2003). On the other side, functional food products commonly have better acceptance among people as a part of the diet. The carrier product should be able to accommodate lutein characteristics, which is heat-labile, sensitive to pH-alteration, well-dissolved and absorbed in oil-based material. The targeted market consists of teenagers to young adults (13–40 years old), health conscious society, especially those who experience prolonged exposure to television, computers, and gadgets. Here, milk-tea was chosen mainly because it belongs to one of the most popular beverages which is well-accepted by all generations. In addition, its opaque brown color might match the yellow color of lutein without inviting specific flavor perceptions of the consumers toward yellow colored beverage (citrus, sour), it also has fat-based ingredients.

Therefore, the objectives of the present study were (i) to perform the solvent-free extraction of marigold petals by using vegetable oil, followed by encapsulation procedure to generate lutein powder; (ii) to incorporate the lutein powder into milk-tea; and (iii) to evaluate the sensory properties of the resulted products. The extracted lutein was transformed into encapsulated form in order to enhance its stability, improve solubility, and ease the application. Moreover, the combination of lutein and milk-tea is intended to create a new salubrious "marimilktea" beverage that meets the need of modern communities.

2. Materials and methods

2.1. Preparation of marigold petals

Marigold flower (*Tagetes erecta* L. var. Mega Orange) was purchased and freshly harvested from the cultivation garden in Badung Regency, Bali, Indonesia. The petals were separated from the stalk prior to drying treatment by means of freeze dryer (Labconco Co., USA) at -45°C under high vacuum condition. 0.04 mbar or vacuum oven (Memmert, Germany) at 40°C , until the moisture content was less than 10%. The dried flower was ground and sieved through a 180- μm sieve. Then, it was stored in low humidity place and protected from light until further use.

2.2. Determination of pigment compositions in marigold petals

One milliliter of acetone was added into 0.01 g of marigold powder for examination of pigments composition. The mixture was vortexed three times, each for 1 min, then centrifuged at 10,000 rpm for 2 min. The crude extract was filtered and subjected into liquid chromatography analysis. The reversed phase C30 (VP-OD) column was eluted with gradient solvent composed of methanol, methyl tertiary butyl ether, and water in high performance liquid chromatography

apparatus coupled with diode array detector (Shimadzu, Japan), in which an aliquot (20 μL) of crude pigment extract was injected. Identification of pigments in marigold's extract was carried out according to Lin, Lee, and Chang (2015).

2.3. Optimization of solvent-free extraction and encapsulation of lutein

Because of the non-polar nature of lutein structure, being bond into one or two fatty acid (known as lutein fatty acid ester or lutein ester), vegetable oil becomes one of the candidates to substitute the use of organic solvent during extraction. Optimization in extraction procedure was carried out in two steps. First, 0.01 g of marigold powder was suspended in 1 mL canola oil, vortexed for 3 min, sonicated for 1 min, and then centrifuged at 10,000 rpm for 2 min. Extraction was performed three times until the color of residue turned pale. Afterward, each supernatant was diluted hundred-fold in hexane and measured spectrophotometrically at 350–600 nm by using Spectrophotometer UV-Vis 1700 (Shimadzu). As the second step, the amount of marigold powder was increased in the series, i.e. 0.03, 0.07, 0.1, 0.2, and 0.3 g/mL oil, and then treated with the same procedure. Quantification of lutein ester was accomplished as acetonic solution with the extinction coefficient published by Jeffrey, Mantrova, and Wright (1997). Recovery value was calculated by comparing the amount of lutein ester extracted in oil to that extracted in organic solvent (acetone and *n*-hexane) as reference. The optimum ratio (0.2 g dried flower per milliliter oil) was chosen to be scaled up and encapsulated for the production of lutein powder.

Moreover, the encapsulation of the pigment extract was done according to the published previous work (Indrawati, Sukowijoyo, Wijayanti, Wijayanti, & Limantara, 2015). Maltodextrin (70 g) and Tween[®] 80 (1.07 g) were dispersed in 105 mL water at 60°C , and 15 mL of lutein extract was added after the mixture cooled down into room temperature. The Litesse[®] (DuPont[™] Danisco[®], France) could be used as replacement of maltodextrin in order to improve the functionality of the beverage by providing prebiotics as well as lowering glycemic load. The suspension was vigorously homogenized at 11,000 rpm (IKA Turrax, Germany) for 3 min, frozen overnight, and subjected to lyophilization (Labconco Co.). Dried material contained less than 10% water, and hence could be ground into vivid yellow powder.

2.4. Milk-tea preparation and incorporation of lutein

The milk-tea was prepared first as the blend of black tea extract (Tong Tji, Perusahaan Teh Dua Burung, Indonesia) and evaporated full cream milk (Frisian Flag, PT Frisian Flag Indonesia, Indonesia). As the topping, liquid whipping cream (Greenfields, PT Greenfields Indonesia, Indonesia) was whisked until it has reached soft peaks. All materials were purchased from local supermarket in Malang. The incorporation of lutein powder could be approached in two manners, either emulsified in milk-tea with the help of Solec[®]F and Recodan[™] (DuPont[™] Danisco[®]), assisted with high-speed homogenizer (Ultra-turrax T-18, IKA Works Inc., USA), or mixed in whipping cream to present a typical yellowish creamy topping. The Xivia[®] (DuPont[™] Danisco[®]) could be added to adjust the final sweetness, while controlling the total calories.

2.5. Focus group evaluation

In the focus group evaluation, small group of consumers were invited to obtain first impression, both positive and negative to the new product, and to provide an early assessment of the prototype. The focus group was comprised of 14 panelists (eight males and six females) who have experience in drinking milk-tea beverage and expressed willingness to participate in the evaluation of "marimilktea". The panelists self-reported that they were in good health. The newly developed beverage was served in three looks, i.e. milk-tea, milk-tea with cream topping, and milk-tea served with cream topping and lutein powder ornamentation (spread above the topping). The lutein powder had been blended in cream portion used in the present evaluation. All samples were three-digit coded using random numbers. The session was conducted between lunch and dinner time. All panelists were asked to value the color, aroma, flavor, mouth-feel, sweetness, and overall preference of the given samples. We determined the consensus result of the focus group and proceeded into hedonic testing.

2.6. Hedonic testing

The milk-tea served with cream topping and lutein powder ornamentation, which was preferred from focus group evaluation, was presented for hedonic rating. The panel comprised of 50 volunteer panelists (10 males and 40 females) of age ranges 17–20 years old (86%), 21–30 years old (8%), and 31–40 years old (6%), which belong to students, academics, and employees of the host University. All panelists were assessed based on personal interest, availability, and having previously consumed milk-tea beverage. The milk-tea was chilled at 7°C overnight prior to sensory evaluation. Each panelist was served 35–40 mL of milk-tea, decorated with 10–15 mL whipped cream. The prototype was coded with random three-digit number and served in clear 50 mL plastic cups. Each panelist was requested to fill out a questionnaire and was asked to rate the color,

aroma, flavor, mouth-feel, sweetness, and overall preference of the given samples using a 9-point hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5-neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, 9-like extremely).

2.7. Color analysis

Color of lutein powder and the prototypes were measured using Color Flex® EZ (HunterLab, USA) and the results were expressed in terms of L^* - lightness, a^* - redness, and b^* - yellowness. The instrument was previously standardized with a white tile ($L = 92.93$, $a = -0.92$, $b = 1.48$). At least two reading per sample was taken and for each reading, the L^* , a^* , and b^* values were recorded.

26 3. Results and discussion

3.1. Pigment composition of marigold petals

Fresh marigold petals contain high amount of water (88%), and hence decreasing water content becomes crucial to prolong the storage time, delay the spoilage, as well as prevent pigments decomposition. The key of pigments preservation is avoiding light irradiation, oxygen, pH change, and high temperature treatment. In the preliminary study, two drying options were selected as the reasonable treatment for further industrial use, which is freeze drying at -45°C and vacuum drying at 40°C (Kurniawan et al., 2017).

Chromatographic profile shown in Figure 1 (left) revealed the dominance of lutein diester in the crude extract of freeze dried marigold petals. After saponification (defatted) treatment, three carotenoids were identified as lutein, violaxanthin, and zeaxanthin (Figure 1, right). Based on peak area calculation, the dominance of *trans*-lutein was 78.15%, followed with 7.15% of violaxanthin and 2.10% of *cis*-zeaxanthin, whereas the remains are epoxy pigments. Total carotenoids content was 9.68 mg lutein/g dry weight in freeze dried marigold and 9.34 mg lutein/g dry weight in vacuum dried treatment (Kurniawan et al., 2017). This value

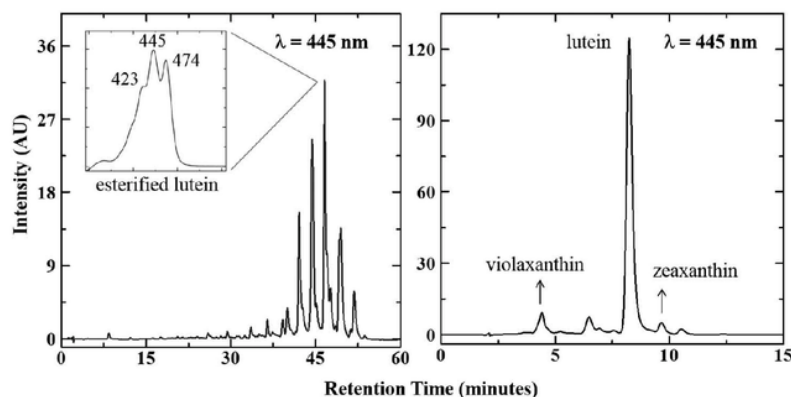


Figure 1. Chromatogram of marigold crude extract in acetonic solution, before (left) and after (right) saponification treatment. Pigments were identified according to previous studies (Lin, Lee, & Chang, 2015; Tinoi, Rakariyatham, & Deming, 2006).

Figura 1. Cromatograma del extracto crudo de caléndula en solución de acetona, antes (izquierda) y después (derecha) del tratamiento de saponificación. Los pigmentos fueron identificados según (Lin, Lee, & Chang, 2015; Tinoi, Rakariyatham, & Deming, 2006).

was comparatively higher than the previously reported by Piccaglia, Marotti, and Grandi (1998) for Italian *Tagetes erecta* and *Tagetes patula*, and being similar to the Chinese marigold species reported by Gao et al. (2010). Thus, this result revealed the potency of marigold locally grown in Bali as the source of lutein.

3.2. Extraction and encapsulation of lutein

In order to determine the extraction capacity of canola oil toward lutein ester, three times of extraction was performed in a certain weight of marigold powder, and then its concentration was gradually increased. The absorption spectrum of lutein was obviously detected in the supernatant of the first and second extraction, giving a typical spectrum of carotenoid with maximum absorption at 443 nm. In the second and third extraction, the intensity was depleted 72.00% and 86.67%, respectively (data not shown), meaning that most pigments had been taken out in the first extraction.

Interestingly, it was found that the quantification of lutein esters in oil-based extraction (6.05 ± 0.02 mg/g) was 190.85% higher than that in acetone (3.17 ± 0.05 mg/g), and being comparable to that in *n*-hexane (7.06 ± 0.09 mg/g). This finding was in line with the result of Gao et al. (2010) which noted that the effectiveness of vegetable oil as a co-solvent in supercritical extraction was nearly same to that of *n*-hexane due to its ability in improving the solubility of lutein esters in the system. Additionally, in order to obtain the optimum ratio between marigold powder and canola oil, a series of concentration was subjected to extraction procedure to give the plot depicted in Figure 2. The concentration of 0.2 g/mL was selected as the optimum ratio for further fabrication of lutein powder.

The encapsulated lutein extract was generated as vivid yellow powder ($L^* - 76.26$, $a^* - 14.58$, $b^* - 86.92$), comprising of 4.27% water content and 41.19 mg lutein esters/100 g powder. The lutein powder was characterized to have better dispersibility in nonpolar environment than water-based system. The presence of lutein in esterified structure is supposed not to influence its bioavailability, since the previous study revealed that bioavailability of lutein esters was comparable to that of free lutein (Wu, Huang, Shi, & Tan, 2009), and no hindrance was possessed upon its conversion into free lutein during clinical trials (Bowen, Herbst, Hussain, & Stacewicz, 2002). The human gut is very efficient in

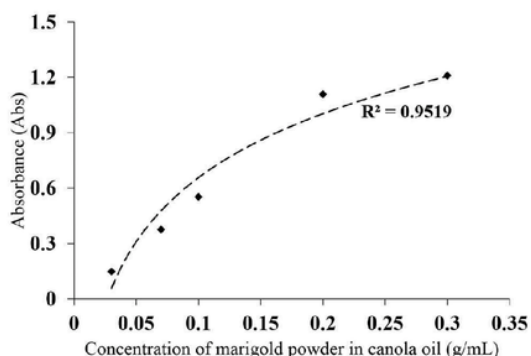


Figure 2. Correlation plot between concentration of dried marigold powder in oil and its absorbance (diluted hundred-fold and measured in *n*-hexane).

Figura 2. Diagrama de correlación entre la concentración del polvo de caléndula deshidratada en aceite y su absorbancia (diluida cien veces y medida en *n*-hexano).

cleaving esters of lutein from its food sources (Bowen & Clark, 2001). However, the adequate amount of fat in our product is more decisive for better bioavailability of carotenoids (Roodenburg, Leenen, van Het Hof, Weststrate, & Tijburg, 2000).

This integrated extraction and encapsulation procedure offers an inexpensive and simple green technology for the production of lutein in the form of solid or powder, which might be applicable for small- to middle-scale local industry. Nevertheless, enhancement of extraction capacity could be attained by using the intensified techniques, i.e. combining the extraction procedure with ultra-sonication, high pressure, or microwave energy (Achat et al., 2012; Hiranvarachat & Devahastin, 2014; Jacotet-Navarro et al., 2016). For the greater and exhaustive industrial scale, the use of supercritical extraction seems to be the most considered option (Boonnoun, Tunyasitikon, Clowutimon, & Shotipruk, 2017).

3.3. Hedonic scores

In the first stage, we conducted a focus group evaluation in order to provide an insight into customer's preference and appraise the critical attributes of this new product. All members of the focus group have had experience in consuming commercial milk-tea beverage before the evaluation. Among the panelists, 71.43% declared that they have consumed milk-tea products more than 10 times throughout life, whereas the remains confessed that they have occasionally consumed it (one to three times).

There are many variations in the serving of milk-tea beverages found in the market. Some popular products in stalls may contain the tapioca pearl or the bursting bubble (known as "boba") made of seaweed alginate with flavored sugar syrup, for example the "Teapresso", "Chatime", "Givetea", "Calais", etc. On the other hand, the teahouses and cafes may also present their milk-tea with the whipped cream as topping, such as the famous Starbucks®, Taiwan Tea House, as well as The Coffee Bean &

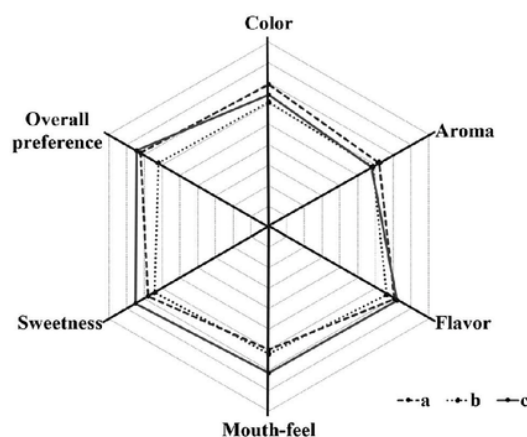


Figure 3. Spider web diagram representing the mean scores for sensory attributes of different serving ways, i.e. milk-tea base (a), milk-tea with lutein-enriched cream topping (b), and milk-tea with lutein-enriched and ornamented cream topping (c). Lutein powder has incorporated into the whipped cream (10 g/L).

Figura 3. Diagrama de telaraña representando las puntuaciones medias de los atributos sensoriales obtenidas según las distintas maneras de servir el producto, por ejemplo, en una base de té con leche (a), té con leche con manto de nata enriquecido con luteína (b), té con leche con manto de nata ornamentado y enriquecido con luteína (c). El polvo de luteína fue incorporado a la nata (10 g/L).

Tea Leaf. In this study, we offered three kinds of serving into the focus group, with and without the whipped cream, and the one spread by some yellow lutein powder above the cream. The presence of whipped cream could be helpful as the fatty material to optimize the solubility of lutein. The result of assessment carried out by the 14 panelists in focus group was depicted as spider chart (Figure 3). Panelists responded the ornamented type more than the others, particularly in color, sweetness, mouth-feel, and overall preference. The presence of yellowish whipped cream, due to the addition of lutein, was well-accepted by the consumers and balancing the overall mouth-feel of the milk-tea.

The product which was chosen by consensus in focus group assessment was brought into hedonic test and introduced as "marimilktea". Figure 4 showed the mean scores for the hedonic test. Most panelists (71%) have experience in tasting the commercial milk-tea beverages about 4–10 times, whereas the remnants have only once impression. Nevertheless, 92% of panelists stated that they like milk-tea beverage. The flavor of "marimilktea" as liked very much (7.84/9.0) by panelists, likewise the sweetness (7.74/9.0), mouth-feel (7.58/9.0), as well as the overall preference (7.54/9.0). The aroma was rated with the lowest score (7.16/9.0), being liked moderately. The typical types and compositions of fat and volatile compounds in whipping cream base were intended to be a crucial factor that influences aroma (Hyvonen, Linna, Tuorila, & Dijksterhuis, 2003; Peterson & Reineccius, 2003).

3.4. Consumer awareness and future prospect of "marimilktea"

During the sensory evaluation, some questions were included into the questionnaire in order to find out the awareness level of consumers toward lutein pigment. Table 1 notified that most consumers neither know marigold flower (60%) nor the benefit of lutein (62%). Among those who understand the lutein, they were able to mention the health benefit of lutein correctly. In the future time, the dissemination of information about lutein and its merit is prospective to improve the consumer preference, as it is applied by most marketing approach.

The incorporation of lutein into milk-tea beverage, named as "marimilktea", is bearing a good prospect. The three main reasons are (i) the milk-tea beverage itself has been well-accepted

Table 1. Awareness of marigold, lutein, and milk-tea beverage.

Tabla 1. Percatación de la caléndula, la luteína y la bebida de té con leche.

| Question | Responses, % n = 50 |
|---|------------------------|
| Have you heard of marigold flower? | |
| Yes | 40 |
| No | 60 |
| Do you know of any health benefits of lutein? | |
| Yes | 38 |
| No | 62 |
| State the health benefits | |
| No response | 66 |
| Prevent age macular degeneration disease | 18 |
| Maintain cognitive health | 4 |
| Antioxidants | 12 |
| Do you know of any health benefits of milk-tea? | |
| Yes | 22 |
| No | 78 |
| State the health benefits | |
| No response | 78 |
| Thirst-quenching drink | 12 |
| Antioxidants | 10 |

by the people, even becomes one of the most trending beverages with growing market potency (Ferruzzi & Green, 2006); (ii) the health benefit of lutein extremely meets the need of modern society toward eyes protection and improved cognitive performance, particularly those who receive longer exposure of radiation from digital display; and (iii) lutein could be obtained in significant amount from *Tagetes erecta* L. var. Mega Orange, an indigenous plant which is locally grown in Bali, Indonesia, through a solvent-free technology, as it has been accomplished in the present study. Additionally, the formulation of "marimilktea" can also be developed, without whipped cream, into instant beverage powder with the appropriate use of suitable ingredients.

4. Conclusions

Lutein pigment, present abundantly in Marigold petals, was successfully extracted in a solvent-free system by using canola oil in the form of lutein esters. The yield of oil-based extraction was almost twice than that of acetone and nearly comparable to that of *n*-hexane, revealing its potency in reducing processing cost while increasing the safety of final product. By using

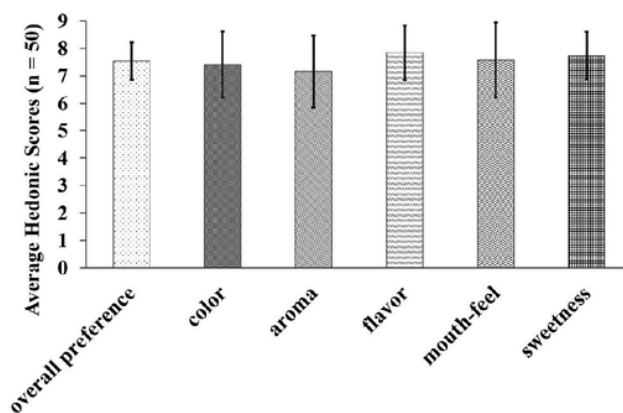


Figure 4. Hedonic scores for the sensory attributes of "marimilktea".

Figura 4. Puntuación hedónica de los atributos sensoriales del té con leche con caléndula llamado "marimilktea" en inglés.

vegetable oil as replacement of organic solvent, the lutein extract could directly be encapsulated to generate the versatile lutein powder. In the present study, the vivid yellow lutein powder was incorporated into milk-tea blend, named as "marimilktea", of which the flavor, sweetness, mouth-feel and overall preference were liked very much by the panelists. The findings of this study might also encourage the use of lutein powder in various food products for added health benefits. However, there is a need for propagation on the health advantages of lutein since more than half of panelists were unaware. Overall, this investigation offered a cheap and feasible process for exploration of marigold flower as an inexpensive indigenous source of the healthful lutein. Improvisation in product formulation and ingredients might also be crucial for future research and development.

Disclosure statement

No potential conflict of interest was reported by the authors.

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