Effect of drying treatments on the contents of lutein and zeaxanthin in orange- and yellow-cultivars of marigold flower and its application for lutein ester encapsulation

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Abstract. Marigold (Tagetes erecta L.) flower is a potential source of lutein and zeaxanthin. The drying treatment of marigold flower is necessary during the industrial process for the preparation of these carotenoids. However, drying treatments with high temperature led the carotenoids undergo the isomerization reaction. Therefore, the aims of this study are to examine the effect of several drying treatments on lutein and zeaxanthin contents, and to characterize a versatility of encapsulated lutein ester powder. Separations of the pigments were performed by high-performance liquid chromatography (HPLC) equipped with photodiode array detector on a C30 column. All-trans isomer of lutein was identified as the major carotenoid of two different marigold cultivars, i.e. Mega Gold (MG) and Mega Orange (MO). The contents of all-trans isomers of lutein and zeaxanthin were 8.95 – 14.55 mg/g dry weight (dw) in MO and 2.56 - 3.73 mg/g dw in MG. The best drying treatment to maintain carotenoid content was freeze drying, while the treatment with oven at 100°C decreased in carotenoids content up to 38%. The encapsulated lutein esters by spray drying and freeze drying showed microparticle size with the different morphological shapes. The encapsulated powders by spray drying produced uniform particles with sperical shape and smooth as well as concave surfaces, while the broken glass of encapsulated powders was obtained by freeze drying. MO cultivar of marigold appears to be a potential source of lutein and zeaxanthin. Encapsulation process may increase in the solubility of carotenoids and widen its application in food or nutraceutical products.

Keywords: drying treatments, encapsulation, lutein, marigold cultivars, zeaxanthin.

1. Introduction
Marigold (Tagetes erecta L.), belongs to Asteraceae family, has been commercially cultivated in several countries for ornament, medicinal, religion and industrial uses. Marigold flower shows white to yellow and orange petal colour indicating the cultivar of flavonoid, essential oil and pigments [1, 2]. These secondary metabolite compounds act as natural insecticide, antibiotic, nematicide and fungicide [3, 4]. The yellow and orange colours of marigold petals are correlated to the presence of carotenoid pigment.
Lutein is the dominant carotenoid and accounts for almost 90%, while zeaxanthin occurs about 5% from the total carotenoids found in the ester forms [5].

Lutein and zeaxanthin are well known as macular carotenoid because both carotenoids together with meso-zeaxanthin are accumulated in macula. Bernstein et al. [6] revealed that 25 carotenoids including lutein have been detected in human milk, serum and tissues. The occurrence of lutein and zeaxanthin in human is strongly related to their roles in human health. Several studies reported the beneficial health effects of lutein and zeaxanthin, namely preventing the age-related macular degeneration disease (AMD) because its function as blue light filter in human eyes [7], acting as antioxidant [8] and anti-inflammation [9], preventing the skin from UV irradiation [10], and increasing the skin health [11]. Nowadays, lutein and zeaxanthin have been reported to have important roles on brain, namely developing the brain and increasing the brain function [12, 13].

Accumulation of lutein and zeaxanthin in the human body is influenced with the daily intake of macular carotenoid-rich foods and lutein supplements. This fact is due to that carotenoid could not be biosynthesized by human. Marigold flower is the potential source of lutein and zeaxanthin compared to the other sources, such as corn, leafy vegetables, etc. [14]. However, the moisture content of fresh marigold petals ranged from 84% to 90% [15] is very sensitive to microorganism activity causing the flower to be rotten [16], although the petals can be stand for 3 days after picking. The drying or removing the water from marigold petals preserved them to have extension shelf-life. Various drying methods such as freeze drying (FD), sun drying (SD), vacuum oven drying (VOD) and conventional oven drying (COD) are common methods in industry and have been applied for the pigment-rich plant materials. FD is a dehydration method that allows the water contained in a frozen material to sublime and gives the highest quality of the final product compared with other drying methods containing heating process, but the cost of FD is higher [17]. The other drying methods, namely SD, VOD and COD are alternative ways, although the drying treatments with high temperature led the carotenoids undergo to the isomerization reaction and tends to have lower bioavailability [18]. Preserving of the carotenoid quality and lowering of the production cost are selection criteria for the suitable drying method.

However, macular carotenoid as well as carotenoids in general have an unstable tendency against environmental factors, i.e. light irradiation, temperature, and oxidation. Their applications on the food and drink products are also limited due to poor solubility in water. Therefore, their bioavailability is low. Encapsulation technology is employed to improve stability and solubility and also to control release of lutein surrounded by coating agents [19]. The most common techniques for encapsulation of bioactive compounds, such as lutein, anthocyanin, phenolic and flavonoid, are spray drying and freeze drying [20-22]. Fast water evaporation, low cost and powder form are the main reasons to use spray drying technique for the food industry [23]. Nevertheless, parameters in spray drying must be optimized especially for encapsulated heat-sensitive bioactive compounds which can be easily decomposed in high temperature. On the other hand, freeze drying is preferably applied to minimize the compound degradation, although the uses of freeze drying have several disadvantages compared to the spray drying.

The study of effect of several drying treatments on the carotenoid in marigold petals is necessary. The effect of drying treatments on the content of lutein and zeaxanthin in local marigold in Indonesia was analysed using UV-Vis spectrophotometer and high performance liquid chromatography (HPLC). Initially, the optimization of extraction solvent was performed to have the best organic solvent for extracting carotenoid from the powder of marigold petals. Besides that, the encapsulation of lutein esters using a mixture of maltodextrin and gum Arabic as coating agents was prepared by spray drying and freeze drying to characterize the morphology of encapsulated lutein ester from the SEM images.

2. Materials and Methods

2.1. Materials

Two cultivars of marigold flowers, namely Mega Orange (MO) and Mega Gold (MG) were harvested from a traditional marigold plantation in Tabanan, Bali.
2.2. Methods

2.2.1. Sample Preparation. The fresh marigold petals of MO and MG cultivars were separated from receptacle and then the petals were frozen (−20°C) until the drying treatments. The petals were dried by 5 different drying methods, namely FD (−45°C, 0.04 hPa), VOD (40°C, 24 hPa), SD (39°C), COD-40 (40°C) and COD-100 (100°C). The petals were dried until the moisture content less than 10%. The dried petals were then ground and sieved with the particle size of 180 μm, and stored at −20°C in the dark.

2.2.2. Carotenoid Extraction and Saponification. The marigold powder (0.01 g) was extracted with different organic solvents (1 mL), i.e. n-hexane (100%), acetone (100%), ethanol (100%) and a mixture of ethanol and n-hexane (4:3, v/v), and then the pigment extracted with the optimum solvent was saponified for HPLC analysis. The extraction process was carried out by vortexing and continued with the centrifugation (14,000 rpm, 2 min, room temperature) to separate the carotenoid extract from the residue. The extraction was repeated until the residue becomes pale and the accumulated supernatants were dried using nitrogen gas (UHP). The saponification of crude carotenoids was performed by dissolving the dried carotenoids in 10 mL diethyl ether (DE) and the pigment solution was added into 10 mL ethanol solution containing 10% potassium hydroxide. The mixture was incubated at room temperature for 2 h in the dark while. The carotenoid in DE layer was washed with water and then carotenoid solution was dried using rotary evaporator (40°C). The dried carotenoid was kept at −30°C in the dark.

2.2.3. Carotenoid Identification and Quantification. The identification of carotenoid from the crude extract and saponified extract from marigold powder was performed by HPLC (Shimadzu, Kyoto, Japan), equipped with photodiode array detector. The sample was dissolved in 1 mL of acetone, filtered using a filter membrane (PTFE, 0.2 μm) and then injected to HPLC with 20 μL of sample loop. Pigment separation was performed on a YMC carotenoid C30 column (150 × 4.6 mm I.D.) with gradient elution program of methanol, methyl tert-butyl ether and water (81:15:4 (v/v/v) at 0 min and 6:90:4 (v/v/v) at 70 min), and the flow rate was 1 mL/min. The identification of the carotenoid was based on the positions of absorption maxima (λmax), the characteristic shape of its absorption spectra and the retention time with comparison to the literature [24]. The content of carotenoid was calculated by using standard curve equation of lutein (y=262.27x+42.92, r² = 0.9997) and zeaxanthin (y=225.14x-159.29, r² = 0.9997) between concentration of carotenoid in μg/mL and peak area at its λmax.

2.2.4. Encapsulation of Lutein Esters. Encapsulation of lutein esters was performed by the modified method of Pal and Bhattacharjee [25]. Lutein esters were dissolved in 3 mL of virgin coconut oil and then dispersed into 100 mL of aqueous solution containing 45 g of wall materials consisted of maltodextrin and gum Arabic (60:40, w/w). The mixture was stirred for 30 mins (450 rpm, Heidolph MR-Hei Standard) at room temperature (25°C) and then continued by homogenization process using IKA Turrax (11,000 rpm, 8 mins, 25°C). The encapsulated product was kept at 4°C in the dark prior to spray drying and freeze drying processes. The spray drying process was carried out using a mini spray dryer (B-290, Buchi, Switzerland) to obtain the powder form of encapsulated lutein esters at following parameters: 160–170°C of the inlet temperature, 0.7 mm of diameter dispersing nozzle, and – 60 hPa of atomization pressure. In the freeze drying process, the solution of encapsulated lutein esters was initially frozen at −30°C for 1 day and then subjected to freeze dry for 48 h at −60°C and 0.032 hPa (Alpha 1-2 LDplus, Martin Christ, Germany). The dried product was ground and then sieved to 150 μm of particle size containing 10.5% of water content. The dried powders of encapsulated lutein esters prepared by spray drying and freeze drying were kept at −30°C.

2.2.5. Characterization of Encapsulated Lutein Esters. The morphology of the encapsulated lutein esters was determined by scanning electron microscope (SEM) (SU3500, Hitachi, Tokyo, Japan). Prior to SEM analysis, the encapsulated powders were sprinkled onto double-side conductive adhesive tape attached on an aluminium specimen stub and then the excess powder was removed by blower. The surface of
encapsulated powders was coated with gold (Au) using an ion sputter coaster (MC1000, Hitachi) under vacuum. The SEM analysis was operated at 5 kV with the working distance of 6.5 mm.

3. Results and Discussions

3.1. Visual Appearance of Dried Marigold Powder

The dried powders of MO and MG cultivars were shown in Fig. 1. According to the visual appearance, the dried powders of MO cultivar were more orange than those of MG. The MO powders obtained by VOD, COD-40 and COD-100 have dark orange colour, while FD showed the bright one. The MO dried by SD was less orange than the others. The visual colour of MG powders obtained from drying marigold petals with SD, COD-40 and COD-100 showed significant colour alteration compared with that of FD which is changed from orange to pale yellow. Effect of drying treatments against marigold colour had been reported by Siriamornpun et al. [26] and Ahluwalia et al. [27]. The colour change of marigold powder was caused by thermal process that leads non-enzymatic browning reaction and degradation of carotenoid inside the marigold. The colour change of marigold powders which turned darker was occurred due to the browning reaction, i.e., MO dried by VOD and COD, MG dried by VOD, while the changes of the powders from orange to pale yellow indicated the degradation of carotenoids [26]. FD was found as suitable drying method for maintaining the colour of marigold compared to the colour of the fresh petals for both cultivars. The colour of marigold powders depends on the concentration of carotenoids, lutein and zeaxanthin. The concentration of these macula carotenoids for each marigold powder will be discussed in the next section.

![Figure 1. Visual appearance of marigold powders with different drying methods, FD (a), VOD (b), SD (c), COD-40 (d) and COD-100 (e).](image)

3.2. Carotenoid Extraction

The absorption spectra of crude carotenoid extracts from the marigold powders extracted with organic solvents and aqueous solutions were shown in Fig. 2. The FD powders of MO cultivar extracted using a mixture of ethanol and hexane (4:3, v/v) showed the highest absorbance value in 1 g dw of powders at its \( \lambda_{\text{max}} \) (158 a.u.), followed by hexane (151 a.u.) and acetone (150 a.u.), while the ethanol extract had the lowest absorbance (116 a.u.) (Fig. 2a). The pattern of these results was in line with the results of MG cultivar (data not shown), however the absorbance values in the absorption spectra of crude carotenoid extracts from MG cultivar were lower compared to those of MO cultivar. The low extractability of carotenoids from marigold powders using ethanol and water (Fig. 2b) is probably due to the occurrence of carotenoid fatty acid esters which have high solubility in oil and non-polar solvents instead of polar solvent and aqueous solution. The absorption around 320-400 nm in absorption spectra of carotenoid extracted with water, ethanol, acetone and a mixture of ethanol and hexane of MO and MG cultivar were related to the absorption of flavonoid compounds [28]. Ethanol and acetone as well as their aqueous solution had been reported as common solvents for extracting the flavonoid from the dried marigold [29, 30]. The absorption of flavonoid compounds may contribute to the absorption of crude carotenoid
extract of marigold. On the other hand, there was no absorption of those compounds in the hexane extract, therefore, the pigment extracted using hexane was chosen as the optimum extract and it will be discussed in the section of carotenoid analysis by HPLC.

![Figure 2](image)

**Figure 2.** Absorption spectra of crude carotenoid extracts from FD-dried MO powders extracted with a mixture of ethanol and hexane (4:3, v/v) (black), 100% ethanol (red), 100% hexane (blue) and 100% acetone (orange) (a), and with water (black) and 100% ethanol (red) (b). The unit of absorbance was calculated in 1 g dry weight (dw) basis.

### 3.3. Carotenoid Analysis by HPLC

HPLC chromatograms of saponified and unsaponified crude carotenoid extracts detected at 445 nm were shown in Fig. 3a. In the saponified extract the dominant peak eluted at 8.2 min was identified as free all-trans lutein, while carotenoid fatty acid esters were detected in between of 38 min and 50 min in the unsaponified extract [5]. Bunea et al. [31] reported that carotenoid fatty acid esters had higher retention time than their free forms when these carotenoids were separated on a reversed phase column. The degree of saturation and length of the fatty acid chains that bind to the carotenoid affects their retention time [31]. The lutein esters contained in the unsaponified crude carotenoid extract of marigold represented almost 95% of total carotenoids, mainly in the forms of lutein laurate-myristate, dimyristate, myristate-palmitate, palmitate-stearate and distearate diesters [32]. De-esterification of lutein esters to the free lutein is performed by saponification process using alcoholic solution, ethanol containing potassium hydroxide [33, 34]. The acylation of lutein does not affect their spectral characteristics. In fact, the absorption spectra of free lutein and lutein esters in hexane had similar $\lambda_{\text{max}}$ at 423, 445 and 473 nm as well as their spectral shape (Fig. 3b). Free- and esterified-lutein could be distinguished by chromatographic measurement instead of spectrophotometry analysis.
Figure 3. HPLC chromatograms detected at 445 nm of unsaponified (red) and saponified (black) crude carotenoid extracts from MO cultivar dried by FD (a) and absorption spectra of the free lutein (black) and lutein esters (red) in hexane (b). Intensity of HPLC chromatograms was calculated in 1 g dw basis, while absorbance value of absorption spectra was normalized at 1.

The separation of free carotenoids from marigold powder could be done less than 11 min using RP-HPLC on C30 column with the gradient elution program of H2O, MeOH and MTBE mixture. Fig. 4a shows HPLC chromatograms of saponified crude carotenoid extracts from MO-FD and MG-FD. These two cultivars of marigold have similar elution profiles with the same peaks such as peaks 1, and 4-8, indicating that those cultivars have the same carotenoid composition. The identification of each peak was according to the chromatographic and spectrophotometric properties as well as the result of co-chromatography analysis with the carotenoid standards. Table 1 summarizes the identification of carotenoids for each peak. Peak 7 was identified as all-trans isomer of lutein which has λmax at 424, 445, and 472 nm and the largest peak area (65 - 85%) compared to other carotenoid peaks. The studies of Hadden et al. [18] and Pratheesh et al. [35] proved that lutein was the dominant carotenoid in marigold flower, while zeaxanthin was one of other minor carotenoids. Peak 8 had λmax at 428, 450, and 476 nm which belong to zeaxanthin. The identification of lutein and zeaxanthin was supported with the results of co-chromatography between saponified carotenoid extract and lutein or zeaxanthin standard (data not shown). Peaks 1 and 4 were confirmed to be violaxanthin and antheraxanthin, respectively, [36]. Peaks 5, 6, and 9 were identified as the cis-isomers of lutein which show the additional cis absorption band at 330-331 nm in their absorption spectra.

HPLC chromatogram of saponified carotenoid extract from MO-COD-100 (Fig. 4b, red colour) showed the additional two peaks, 2 and 3, which can be identified as cis-isomers of carotenoid due to the presence of cis absorption band. In addition, Fig. 4 left (insert) indicated that cis-isomer peaks, 2, 3, 5, and 6, were formed and significantly increased after the drying treatment with the COD at 100°C. On the contrary, the peak area of all-trans isomers of carotenoids decreased especially for the peak of all-trans isomer of lutein. The isomerization mechanism of all-trans isomer of lutein into its cis-isomers was proposed by Updike and Schwartz [37]. In addition, the isomerization or degradation of carotenoid is known to be caused by the thermal oxidation. The drying with COD-100 on the marigold flower totally degrades the violaxanthin and antheraxanthin. It is reported that violaxanthin and antheraxanthin as the epoxy-carotenoid group degraded faster due to the formation of furanoid caused by epoxide isomerization [38]. The ester forms of hydroxyl-carotenoids, lutein and zeaxanthin contained in marigold flower were more stable in the heat treatment compared to the free from of carotenoids.
Figure 4. HPLC chromatograms detected at 445 nm of saponified carotenoid extracts of MO (black) and MG (red) cultivars of marigold dried by FD (a), and MO-FD (orange) and MO-COD-100 (blue) (b). The intensity unit was calculated in 1 g dw.

Table 1. Identification of carotenoids from saponified carotenoid extracts of MO and MG based on the retention time and \( \lambda_{\text{max}} \) in HPLC solvent.

| Peak number | Retention time [min] | \( \lambda_{\text{max}} \) [nm] | Identification* |
|-------------|----------------------|---------------------------------|------------------|
| 1           | 4.4                  | 416, 439, 469                   | Violaxanthin     |
| 2           | 5.2                  | - , 432, 454                    | cis-isomer of carotenoid |
| 3           | 5.6                  | - , 433, 456                    | cis-isomer of carotenoid |
| 4           | 6.5                  | - , 421, 444, 470               | antheraxanthin    |
| 5           | 6.9                  | 331, 421, 439, 466             | 13- or 13'cis isomer of lutein |
| 6           | 7.6                  | 330, 422, 438, 464             | 13- or 13'cis isomer of lutein |
| 7           | 8.3                  | - , 424, 445, 472              | all-trans isomer of lutein |
| 8           | 9.7                  | - , 428, 450, 476              | all-trans isomer of zeaxanthin |
| 9           | 10.5                 | 330, - , 441, 468              | 9- or 9'cis isomer of lutein |

Note: * refer to Gupta et al. [36] and Updike and Schwartz [37].

The contents of lutein and zeaxanthin were calculated using the equations of standard curves \( (r^2 = 0.9997) \) for lutein and zeaxanthin which are expressed as mg of carotenoid in 1 g dw. The contents of lutein and zeaxanthin in MO and MG cultivars obtained by several drying methods are shown in Table 2 and Fig. 5. The contents of lutein and zeaxanthin of MO cultivar dried by FD from crude carotenoid extract prepared by extraction with 100% hexane were 13.94 mg/g dw and 0.61 mg/g dw, respectively. These carotenoid contents were higher compared to the same sample extracted with a mixture of ethanol and hexane (4:3, v/v) which was having 9.68 mg/g dw of lutein and 0.40 mg/g dw of zeaxanthin. Hexane had been used as extraction solvent of carotenoids from the powder of marigold petals [39]. Recently, Boonnoun et al. [34] evaluated the extraction conditions on the yield of lutein esters from the dried marigold using extractions of hexane, liquefied DME and SC-CO\(_2\). The hexane extraction had higher yield of lutein esters compared to that of SC-CO\(_2\), although DME extraction produced the highest yield.

The lutein content in MO and MG cultivars were 25 and 15 times higher than those of zeaxanthin, respectively. Moreover, the total carotenoids, contents of lutein and zeaxanthin, in MO cultivar was almost four times higher than those of MG. This result was in line with experimental report of Piccaglia et al. [40] dealing with the colour of marigold flower depends on the carotenoid content. Lin et al. [41]
also identified the sub species of *Tagetes erecta* by the colour, namely yellow, orange or brown which is indicated with the different amounts of lutein. Lutein content increases as the marigold colour intensifies becoming more orange. Li *et al.* [42] and Ingkasupart *et al.* [2] reported about the lutein and lutein ester contents in 11 marigold cultivars grown in Thailand and in China ranging from 8.31 to 20.59 mg of lutein/g dw and 1.61 to 6.11 mg of lutein esters/g dw, respectively. The lutein content of marigold from Thailand is in the range to that of MO cultivar of local marigold from Indonesia (8.61 – 13.94 mg/g dw; Table 2), while the content of lutein esters of marigold cultivars from China was lower 1.4 to 8.7 times. Thus, MO cultivar of Indonesian local marigold has high potency as the source of lutein.

**Table 2.** Lutein and zeaxanthin contents in MO and MG cultivars of marigold flower dried by several drying methods.

| Drying Method | Carotenoid Content [mg/g d.w. ± SE] |  |  |
|---------------|----------------------------------|---|---|
|               | Mega Orange | Mega Gold | Mega Orange | Mega Gold |
| FD            | 13.94 ± 0.62 | 3.51 ± 0.21 | 0.61 ± 0.06 | 0.22 ± 0.01 |
| VOD           | 13.23 ± 0.12 | 3.24 ± 0.21 | 0.60 ± 0.05 | 0.22 ± 0.02 |
| COD-40        | 12.46 ± 0.21 | 3.15 ± 0.14 | 0.44 ± 0.01 | 0.20 ± 0.01 |
| SD            | 10.46 ± 0.60 | 2.55 ± 0.23 | 0.40 ± 0.02 | 0.16 ± 0.02 |
| COD-100       | 8.61 ± 0.33  | 2.42 ± 0.21 | 0.34 ± 0.02 | 0.14 ± 0.01 |

**Figure 5.** Bar charts of lutein (a) and zeaxanthin (b) contents (mg/g dw) from MO cultivar (red) and MG cultivar (orange) of marigold flower dried by FD, VOD, COD-40, SD and COD-100. Carotenoid contents are averaged of 3 replications and bars represent standard error.

Fig. 5 shows the comparison of lutein and zeaxanthin contents from MO cultivar and MG cultivar of marigold after drying by different methods. The dried marigolds by FD and VOD had higher lutein and zeaxanthin contents compared to other drying methods. There was no significant difference on the contents of lutein and zeaxanthin between both methods, indicating that FD and VOD were suitable method for maintaining the colour of dried marigolds as well as the carotenoid content. This result is in agreement with previous results of Siriamornpun *et al.* [26] and Ahluwalia *et al.* [27] that showed less colour alteration and higher bioactive compounds contained after drying the marigold with FD and VOD method. As drying method, COD-40 and SD can be used as alternative methods because of the economic
reasons, but these drying methods required longer time to reach the water content less than 10%. The least drying method, COD-100, decreased the lutein and zeaxanthin contents up to 38% for MO and 30% for MG. In addition, thermal processing of marigold by COD-100 lead the carotenoid isomerization. Therefore, COD-100 was not suitable for drying the marigold flowers.

3.4. Characterization of Encapsulated Lutein Ester

SEM micrographs of the encapsulated lutein esters obtained by spray drying and freeze drying with magnification of 1000 and 100, respectively, are shown in Fig. 6. Fig. 6a shows that particles of encapsulated lutein ester resulted by spray drying were formed in the heterogeneous forms. The particles with different sizes including large particles are correlated to agglomeration [43] and produced using maltodextrin as coating agent [44], while Ferrari et al. [44] revealed that small particles of encapsulated powders were obtained with gum arabic. Particle sizes of the encapsulated powders were in the range of 1.12–24.80 µm (mean of particle diameter = 9.56 µm). The morphology of encapsulated powder showed spherical shape and smooth outer surface, although there was a concavity as the result of shrinkage during rapid water removal process, related to the temperature of gas inlet that is a parameter used in the spray drying. The result by morphological observation was similar with Pal and Bhattacharjee [25] and Ferrari et al. [44] which reported the spray drying of encapsulated lutein and anthocyanin, respectively, using maltodextrin and gum arabic as wall materials. The usage of different wall materials, such as maltodextrin and β-cyclodextrin, for spray drying also showed the same morphology with concave surface, i.e., without cracks or pores Papoutsis et al. [22]. Encapsulation of lutein with copovidone at 100–120°C as the temperature inlet produced smooth and spherical microparticles [19]. Therefore, the formation of concavities in the particle surface is not associated with the type of wall material used, but depends on the spray-drying parameters. In contrast, the encapsulated powders obtained by freeze drying process showed a broken glass or flakes-like form, where the maximum size of the particles was less than 150 µm due to the sieving process (Fig. 6b). This structure is related to the process of sublimation of the water contained under low temperature, so there is no force to produce the droplet [22].

**Figure 6.** SEM micrographs of encapsulated lutein ester containing maltodextrin and gum arabic prepared by spray drying with magnification of 1000 (a) and freeze drying with magnification of 100 (b).

4. Conclusions

The effect of drying treatments for marigold flower on the lutein and zeaxanthin contents was varied. The FD and VOC have the highest content of both carotenoids followed by COD-40 > SD > COD-100. MO and MG cultivars of marigold flower have the same pattern on carotenoid contents against drying.
treatment. Lutein was the dominant carotenoid in MO cultivar dried by FD containing 13.94 mg/g dw which was extracted with 100% hexane, while the zeaxanthin content is almost 22.1–28.3 times and 14.7–17.3 times lower compared to those of lutein in MO cultivar and in MG cultivar, respectively. SEM analysis of encapsulated lutein esters by spray drying showed that the powders had spherical shape with uniform sizes of micro particles (1.12–24.80 μm), while the encapsulated powders prepared by freeze drying showed the broken glass shape with the particle sizes less than 150 μm.

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