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Production of monodisperse epigallocatechin gallate (EGCG) microparticles by spray drying for high antioxidant activity retention

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ABSTRACT

Epigallocatechin gallate (EGCG) originated from green tea is well-known for its pharmaceutical potential and antiproliferating effect on carcinoma cells. For drug delivery, EGCG in a micro-/nanoparticle form is desirable for their optimized chemopreventive effect. In this study, first time reports that EGCG microparticles produced by low temperature spray drying can maintain high antioxidant activity. A monodisperse droplet generation system was used to realize the production of EGCG microparticles. EGCG microparticles were obtained with narrow size distribution and diameter of 30.24 ± 1.88 μM and 43.39 ± 0.69 μM for pure EGCG and lactose-added EGCG, respectively. The EC50 value (the amount of EGCG necessary to scavenge 50% of free radical in the medium) of spray dried pure EGCG particles obtained from different temperature is in the range of 3.029–3.075 μM compared to untreated EGCG with EC50 value of 3.028 μM. Varying the drying temperatures from 70 °C and 130 °C showed little detrimental effect on EGCG antioxidant activity. NMR spectrum demonstrated the EGCG did not undergo chemical structural change after spray drying. The major protective mechanism was considered to be: (1) the use of low temperature and (2) the heat loss from water evaporation that kept the particle temperature at low level. With further drier optimization, this monodisperse spray drying technique can be used as an efficient and economic approach to produce EGCG micro-/nanoparticles.

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1. Introduction

Epigallocatechin gallate (EGCG) as the most abundant catechin in green tea leaves has been widely acknowledged for its promising beneficial effects on health (Huo et al., 2008). EGCG can be administered via oral and intravenous routes (Paolino et al., 2007). Besides these two routes, it can also be delivered to gastrointestinal track through intraperitoneal administration (Giakoustidis et al., 2006). EGCG exhibits antioxidant (Kurisawa et al., 2004), anti-bacterial (Maeyama et al., 2005), and anti-inflammatory (Trompezinski et al., 2003) activities. EGCG can also reduce fat absorption and reduce the risk of cancer and cardiovascular diseases (Han et al., 2010). Extensive research has been devoted to investigate the pharmaceutical potential of EGCG, in particular, on the inhibition of carcinogenesis and tumour growth (Jung et al., 2001; Lu et al., 2002). However, EGCG as a polyphenol is susceptible to oxidation (Hosny and Rosazza, 2002). The potentially low bioavailability and short half-life of polyphenols limit their therapeutic application. One of the approaches to overcome this hurdle is to modify EGCG particle with coating, the coating can add functionality as well as conferring the particle with physical and chemical protection. Particle coating is often used to modify the surface of spray-dried molecules (Elversson and Millqvist-Fureby, 2006; Shutava et al., 2009; Siddiqui et al., 2009). Various attempts have been made to improve the bioavailability of EGCG using other approaches including the use of polylactic acid–polyethylene glycol (PLA–PEG) microencapsulation (Siddiqui et al., 2009), monophasic lipid co-solubilization (Smith et al., 2010), polyphenol/protein binding (Shpigelman et al., 2010) and layer-by-layer coating (Shutava et al., 2009). These methods generally require complex formulation and the procedures can be time-consuming. In addition, it can be diffi-

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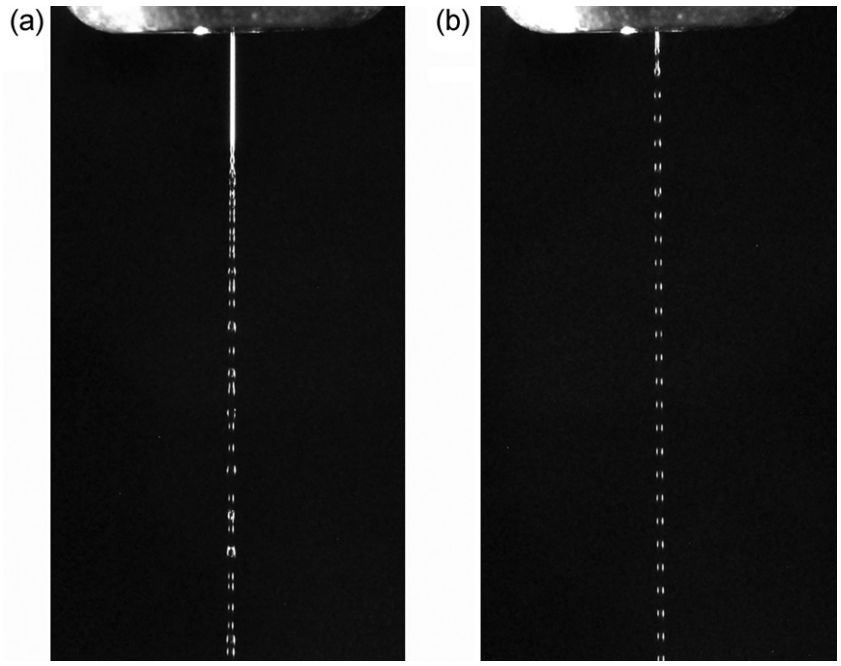

Fig. 1. Monodisperse droplet generation. (a) Stream of feed solution from the nozzle before applying the electric signal, and (b) stream of feed solution consisting of monodisperse droplets, obtained with the optimized electrical frequency.

cult to control the size and functionality of EGCG particles produced by some of these methods (Shutava et al., 2009).

Spray drying is a common approach to produce powdered products in food and pharmaceutical industry. It is feasible to produce powders of heat-sensitive materials using spray dryer, if the residence time is short enough to prevent the material from heat induced deterioration (Broadhead et al., 1992; Mumenthaler et al., 1994). Spray drying process has been used to prepare inhalation particles for pulmonary drug delivery (Kawakami et al., 2010), and is particularly useful for microencapsulation of core material with a protective shell in a single step (Ilic et al., 2009). During conventional spray drying, the feed solution is dispersed into droplets, which enter the drying tower together with a concurrent hot air stream. The tower is usually heated to temperature well above boiling point of water to dry the water quickly from the droplet. The final particles generated in such process are often of large size distributions, ranging from 5 μm to 250 μm in diameter, due to the differences in the initial droplet size after dispersion (Ilic et al., 2009; Kawakami et al., 2010). Droplets with different initial sizes undergo different drying histories in the drier (Patel and Chen, 2007; Wu et al., 2007). For drying materials that are heat and/or oxygen sensitive, the differences in the changing histories of droplet temperature and moisture content will lead to varied residual activity in individual particle. It has been reported that the different initial droplet sizes affect the maintenance of enzyme activity and the degree of protein denaturation during convective drying processes (Vega and Roos, 2006; Yamamoto and Sano, 1992). As a result of the different droplet/particle sizes, the particles obtained from conventional spray drying can have different residual activity, and therefore resulted in large variation in quality. For pharmaceutical usage and drug delivery, powders with large size distribution and large variation in bioactivity are undesirable (Shutava et al., 2009).

Recently, a monodisperse droplet generator was designed and equipped on spray driers as atomiser (Wu et al., 2011b). The droplets generated by this type of atomiser have monodisperse size, thus undergo similar drying histories when they are drying in the same conditions (e.g., flow rate, temperature). In this study, different spray driers were equipped with atomiser (monodisperse droplet generator) to investigate the feasibility of producing EGCG-microparticles by spray drying. EGCG microparticles with uniform size and with high retention of antioxidant activity were obtained.

2. Materials and methods

2.1. Monodisperse droplet generator and spray driers

The structure of the monodisperse droplet generator has been described in detail elsewhere (Wu et al., 2007, 2011b). Briefly, the feed solution was placed in a 1.5 L reservoir, and the reservoir was connected to a compressed air supply which force the feed solution to pass through a glass nozzle that act as an atomiser. The glass nozzle contained a glass capillary tube with an orifice of 50 μm, surrounded by zirconate/lead titanate ceramics (APC International Ltd., Mackeyville, PA, USA). The piezoelectric ceramic component was connected to Jet Drive III pulse controller (Microfab Technologies Inc., Bedford, MA, USA). In order to produce monodisperse droplets, the pulse controller generated a sinusoidal electrical signal with a controllable frequency, which worked on the piezoelectric ceramic component and generated a pulse on the feed solution stream that pass through the glass nozzle. The piezoelectric pulsing was adjusted to a frequency range of 7000–15000 Hz for droplet generation optimization. A photograph of the monodisperse droplet stream resulted from atomisation is shown in Fig. 1.

To test the feasibility to producing EGCG microparticles with high activity retention by spray drying, two spray driers, namely mini drier and big drier, equipped with the monodisperse-droplet-generating nozzle were employed. Schematic illustration of these two driers is shown in Fig. 2. The mini drier with a cuboid body was connected to a pyramidal outlet. The compressed air supply was regulated using a pressure regulator (SMS Pneumatics Pty Ltd., Australia). The air was heated by a set of electric elements before entering the drier. The mini drier has shorter height (980 mm) compared to the big drier (3000 mm), and is designed for production of smaller particles (≤10 μm) that can lose moisture within a short time. The shorter height results in shorter residence time which can

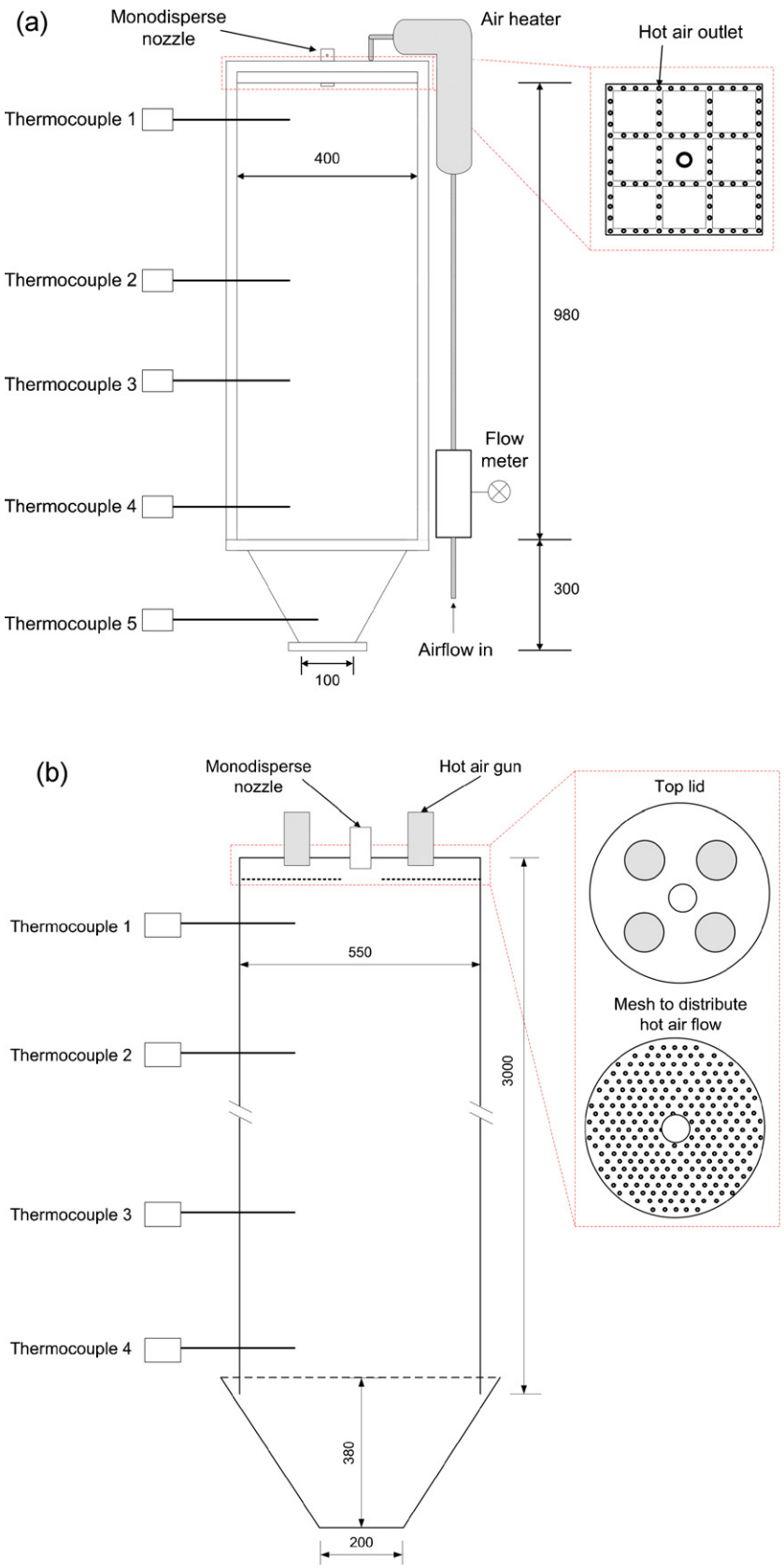

Fig. 2. Schematic illustration of the two driers used in the current study (not to scale in mm). (a) Mini drier, and (b) big drier.

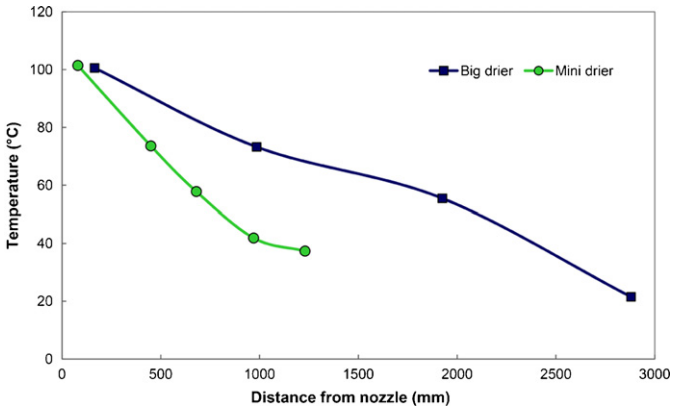

Fig. 3. Temperature profiles of the two driers with an inlet temperature of 100 °C (process value).

prevent particles from over-heating. The big drier has a cylindrical body with a conical outlet. A concurrent hot air flow was generated by four hot air guns (Robert Bosch Pty Ltd., Australia). In order to achieve evenly distributed air flow, the hot air passed though an air-dispersion mesh plate before entering the drying tower. The big drier with taller height is designed for production of larger particles (e.g., 100 μm), as it provides sufficient residence time for drying process to complete. Both driers were installed with several thermocouples along the drier body to measure the temperature profile inside the drier (Fig. 2). For convenience, the set temperature of each run will be referred as drying temperature.

The mini drier equipped with a temperature controller (Eurotherm Pty Ltd., Ashburn, VA, USA) allows the desired temperature of inlet air stream to be set.

For the big drier equipped with four air guns, the temperature was set by adjusting the outlet temperature of the air guns. The actual inlet temperature was measured by the thermocouple at the entrance of drier. It should be noted that the actual inlet temperature was different from the set value in both driers. Fig. 3 shows the actual temperature profiles along the two driers, where mini drier temperature was set at 95 °C and big drier temperature was set at 130 °C. The temperature and other process parameters used in the current study are listed in Table 1.

2.2. Feed solution and powder storage

Two feed solutions were examined in this study. The first solution contained pure EGCG reconstituted from Teavigo® (DSM Nutritional Products Ltd., Switzerland) at a concentration of 1% (w/v) in Milli-Q water (Milli-Q system, Millipore Australia Pty Ltd., Australia). The second solution contained 1% (w/v) EGCG and 4% (w/v) lactose (Sigma L8783, Sigma–Aldrich, Australia) in Milli-Q water. Lactose is a common excipient use in pharmaceutical industry (Takeuchi et al., 2000). In this study lactose was employed as a model coating material, attempt was made to investigate whether the activity of EGCG will be affected by a coating material. All feed solutions were prepared under O_2 free atmosphere with continuous purging of nitrogen gas to prevent EGCG from oxidation. Powders obtained after spray drying was purged with nitrogen and stored at −18 °C freezer before further analysis.

2.3. Moisture content of the spray-dried powders

To estimate the moisture content of spray-dried EGCG microparticles, an aliquot of spray-dried powders were oven dried at 104 °C for 2 h to remove residual moisture content. The samples were weighed both before and after drying using an analytical balance (Sartorius AG, Germany).

2.4. Scanning electron microscopy (SEM) observation

Scanning electron microscopy (SEM) was used to visualise spray-dried particles' morphology and to determine particle size. The particles obtained from the spray drier were fixed to an aluminium sample stub using conducting carbon tape followed by sputter-coating with ~1 nm gold–palladium to produce a conductive surface for SEM observation. Secondary electron SEM images were recorded using a JEOL 840A (Jeol Co. Ltd., Japan) operated at 5–15 kV.

2.5. Nuclear magnetic resonance (NMR) analysis

Nuclear magnetic resonance (NMR) spectroscopy was used to examine structural changes in EGCG after spray drying. Both untreated and spray dried EGCG were analysed by ^{13}C and ^{1}H liquid state NMR with D_2O as carrier. NMR experiments were collected on Bruker Avance400 (9.4 Tesla magnet) with a 5 mm broadband autotunable probe with Z-gradients at 30 °C. The spectra were collected and processed using Brukers Topspin 2.1 program.

2.6. DCFH-DA assay

DCFH-DA assay (Brown et al., 2006) was used to estimate the loss of antioxidant activity in spray dried EGCG. DCFH-DA reagent was prepared by dissolving 12.2 mg of 2′,7′-dichlorfluorescin diacetate (DCFH-DA) (Sigma) in 25 ml of absolute ethanol. H_2O_2 stock solution with 750 mg/L concentration was prepared. EGCG samples were reconstituted at a concentration of 1% (w/v) in Milli-Q water. The H_2O_2 stock solution was diluted to 1:5, 1:10, 1:20, 1:50 ratio using Milli-Q water, corresponding to a H_2O_2 concentration of 150, 75, 37.5 and 15 mg/L. DCFH-DA reagent, H_2O_2 reagent, and EGCG samples were mixed at a proportion of 66.64 μL, 133.36 μL and 40 μL, respectively. The mixture was incubated in the dark for 30 min before subject to fluorescence reading using a microplate reader (SpectraMax M2, Molecular Devices, USA). The excitation and emission wavelength were 485 and 528 nm, respectively.

Table 1
Process parameters used for the two driers.

	Mass spray rate (g/min)	Average electrical frequency (Hz)	Set T_a (°C)[a]	Process T_a (°C)[a]	
				Inlet	Outlet
Mini drier	0.55 ± 0.2	14,000	95	101	37
			105	112	45
Big drier	0.80 ± 0.5	9000	130	100	22
			110	87	22
			90	72	22
			80	68	22
			70	64	22

[a] T_a: air drying temperature (°C).

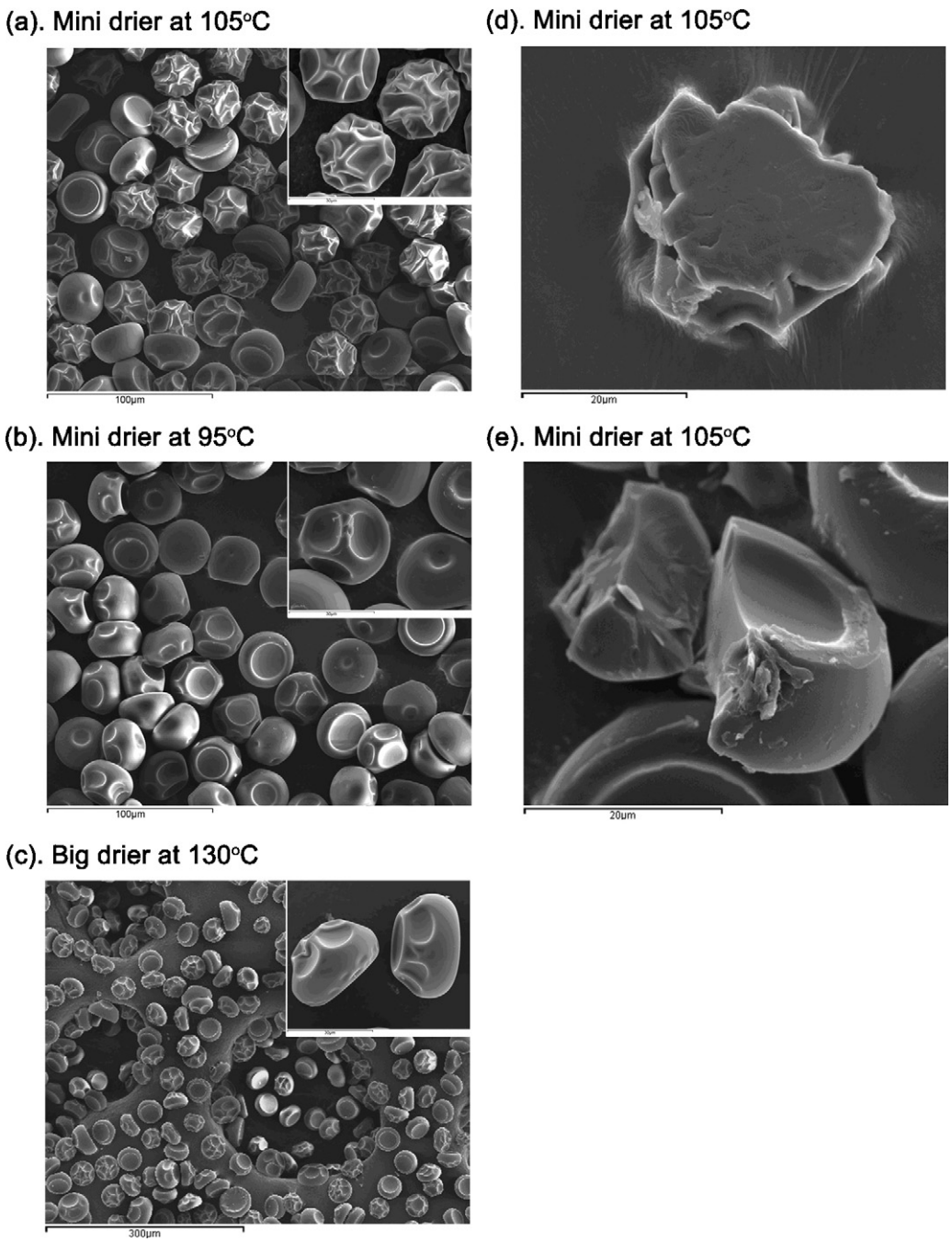

Fig. 4. SEM pictures of pure EGCG microparticles. (d and e) show the interior of microparticles dried in the mini drier at set temperature 105 °C.

Fresh EGCG was used as Positive Control 1. During spray drying, air is used to drive the feed solution through the nozzle; the feed solution from the reservoir is therefore constantly flushed by air and may loss some activities due to oxidation. To determine the activity loss caused by the air flush, residual EGCG feed solution in the reservoir was collected at the end of spray drying and was subject to assay as Positive Control 2. Negative control of EGCG was obtained by drying fresh EGCG solution at a 60 °C oven overnight. EGCG is susceptible to oxidation, therefore identical EGCG sample that subjected to assay on different day may give different readings. In order to reduce error arise from sample variability, on top of running replicate samples, fresh controls and fresh spray dried samples were prepared on a daily basis. Although different readings might be obtained if assay were conducted on different day, a correct trend can be obtained by comparing fresh samples to fresh controls.

2.7. DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical. Upon mixing with a hydrogen donor such as active EGCG, the colour of DPPH solution will turn from violet to pale yellow. DPPH scavenging activity was determined using a modified method of Brand-Williams et al. (1994). In brief, 50 μL of various dilutions of EGCG particle were mixed with 1 ml of 60 μM 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma) in methanol. After an incubation period of 30 min, the optical density (OD) of 200 μL of sample aliquot was read at 517 nm using a microplate reading. Assay con-

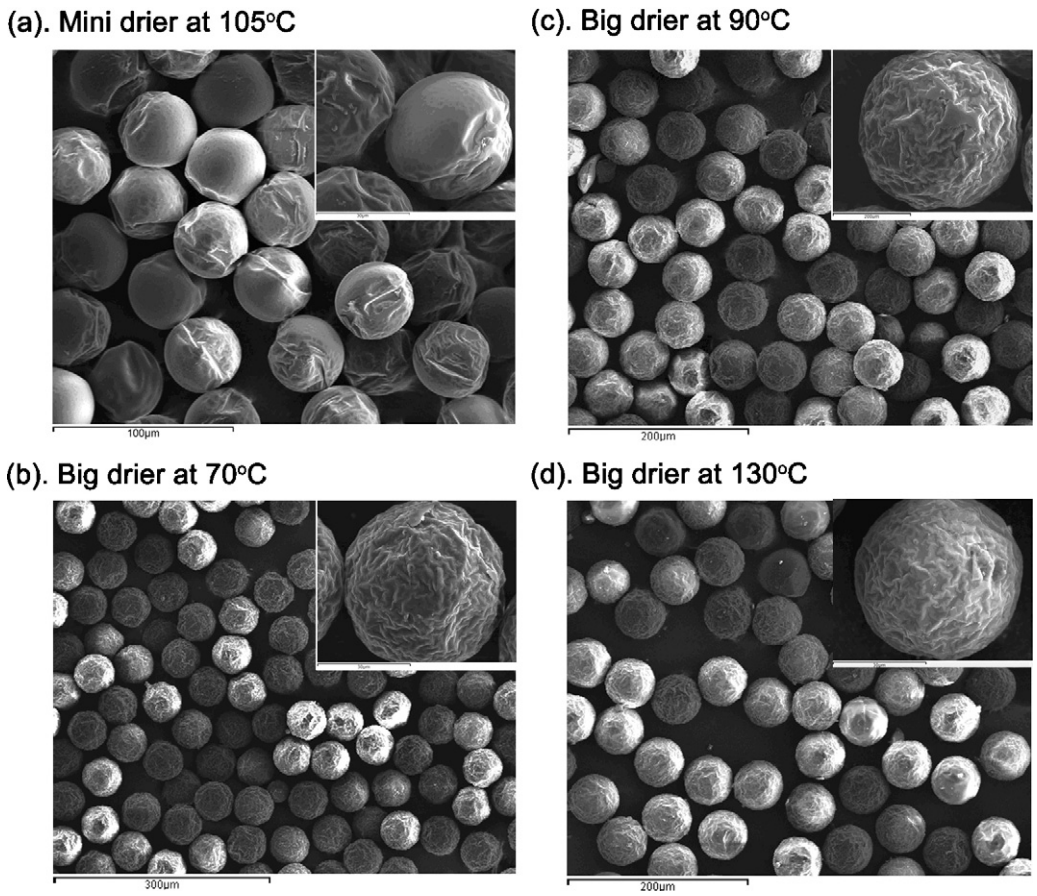

Fig. 5. SEM pictures of lactose-added EGCG microparticles.

trol (AC) consisted of 50 μL of methanol and 1 ml of DPPH solution. The DPPH radical stock solution and samples were prepared fresh daily. The DPPH radical scavenging activity of EGCG at each dilution was calculated by:

$$\text{Radical scavenging activity}(\%) = \frac{\text{OD}_{\text{AC}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{AC}}} \times 100\% \qquad (1)$$

The results were used to calculate the EC50 (the amount of EGCG necessary to decrease the initial DPPH concentration by 50%) of EGCG on superoxide radical scavenging. EC50 of spray dried EGCG was compared to that of untreated EGCG (positive control 1), residual EGCG feed solution in the spray dryer reservoir (positive control 2), and oven dried EGCG (negative control). A lower EC50 value represents a higher antioxidant activity.

3. Results

3.1. Particle size, morphology and moisture content of spray-dried EGCG microparticles

The properties of spray dried EGCG and their corresponding conditions are listed in Table 2. The morphology of pure EGCG

Table 2
Drying conditions and particle properties of the spray-dried EGCG microparticles.

Feed solution	Drying conditions		Particle properties	
	Drier	Set T_a (°C)[a]	D (μm)/($D \times h$) (μm)[b,c]	u (kg/kg)[b] (%)
1% EGCG (w/v)	Mini drier	95	$(32.48 \pm 0.48) \times (18.18 \pm 1.96)$	~7.4
	Mini drier	105	(30.24 ± 1.88)	~4.0
	Big drier	130	$(36.72 \pm 2.83) \times (20.35 \pm 1.58)$	~2.5
1% EGCG & 4% lactose (w/v)	Mini drier	105	43.39 ± 0.69	~10.9
	Big drier	70	55.95 ± 0.54	~9.0
		80	55.72 ± 0.98	~6.5
		90	54.99 ± 0.83	~6.5
		110	55.38 ± 0.79	~4.2
		130	54.89 ± 0.66	~3.9

[a] For mini drier, T_a was set by a PID controller to control the temperature of inlet air flow into the drier. For big drier, T_a was set by adjusting the outlet temperature of the four hot air guns.
[b] D: particle diameter (μm); h: particle thickness (μm); u: particle moisture content (kg/kg).
[c] Particle size was determined using SEM. For particles in a disc shape that cannot be considered a sphere, the diameter of the circular plane and the thickness of the particle were measured. The uncertainty shown was the standard deviation of at least 10 particles measured.

and lactose-added EGCG microparticles are shown in Figs. 4 and 5, respectively. Particles obtained within the same batch had a fairly similar size distribution, indicating that monodispersed droplets can be obtained using both mini drier and big drier. Spray dried EGCG particles obtained from the mini drier had similar particle diameter regardless of the set temperature. For example, the diameter of EGCG particles obtained at set temperatures of 95 °C was 32.48 ± 0.48 μm, while the diameter of EGCG particles obtained at set temperature of 105 °C was 30.24 ± 1.88 μm. Whereas lactose-added EGCG particles obtained from mini drier had larger diameter (43.39 ± 0.69 μm) due to the higher solid content in the feed solution (5% (w/v) compared to 1% (w/v)).

Regardless of the composition, particles obtained from big drier had larger diameter compared to that obtained from mini drier (Table 2). The difference in particle size could be a result of different initial droplet size generated from the different drier, although nozzles in both drier were of the same size (orifice: 50 μm). The reason for this observation and its impact on the maintenance of EGCG activity will be discussed later in Section 4.2.

In terms of particle morphology, pure EGCG microparticles obtained from mini drier at 105 °C exhibited two distinctly different morphologies (Fig. 4a). One of the particle populations exhibits biconcave morphology, while the other population exhibits spherical morphology with corrugated surface. SEM images showed that both spherical (Fig. 4d) and biconcave (Fig. 4e) particles had solid cores. Pure EGCG microparticles obtained from mini drier at 95 °C exhibited convex-concave morphology with corrugated edge of the convex side (Fig. 4b), which is similar to the morphology of one of the populations obtained at 105 °C. Pure EGCG microparticles obtained from big drier exhibited plano-convex morphology with corrugated edge on the convex side.

The use of lactose as EGCG coating material was subject to spray drying, the addition of lactose increased the overall size and weight of particles due to the increase in overall concentration in the feed solution. The addition of lactose will produce larger and heavier particles, which are comparatively easier to collect in both the big and mini drier. Spray drying was carried out using the big drier at different drying temperatures (70 °C, 80 °C, 90 °C, 110 °C and 130 °C) to investigate the potential effect of inlet temperature on the EGCG microparticles' properties. As shown in Fig. 5, the variation in the drying temperature showed little effect on lactose-added EGCG particle morphology, dried particles obtained at different temperature exhibited spherical morphology with corrugated surface (Fig. 5b–d). Spray dried lactose-added EGCG exhibited spherical shape with corrugated surface; this morphology is similar to that of pure lactose. Pure lactose was reported to undergo even shrinkage during drying and yield spherical particles (Wu et al., 2011a). Lactose-added EGCG microparticles obtained from mini drier exhibited spherical shape as shown in Fig. 5a, while one hemisphere appeared smooth and the other appeared corrugated. This feature indicates the drying is not even, possibly due to the shorter residence time. As a result of shorter residence time, lactose-added EGCG microparticles obtained from mini drier had higher residual moisture content up to 10.9% (kg/kg) compared to those obtained from big drier at the similar set temperature.

Moisture content of the dried EGCG particles was higher at lower drying temperatures. When drying temperature was increased from 95 °C to 105 °C, the moisture content of pure EGCG particles decreased from 7.4% to 4% kg/kg, indicating that the drying was more complete at higher drying temperatures (Fu and Etzel, 1995). Pure EGCG particles dried in the big drier exhibited the lowest moisture content of around 2.5%, which could be due to two reasons: (1) the higher temperature profile in the drying tower (Fig. 3) and (2) the longer residence time inside the drying tower. In the big drier, decrease in drying temperature also results in particles with higher moisture content.

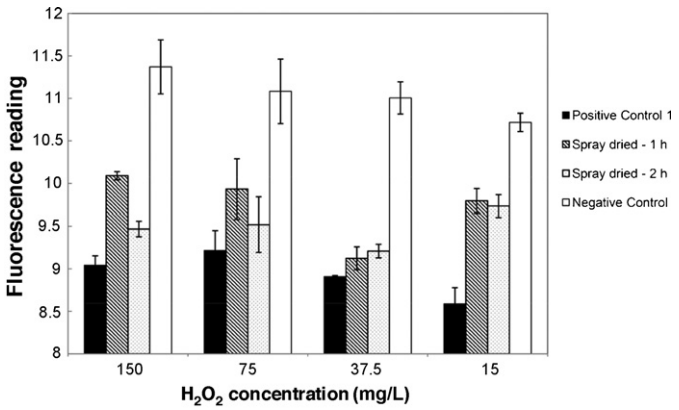

Fig. 6. H_2O_2 scavenging activities of spray-dried EGCG microparticles obtained from big drier (set temperature 130 °C) were evaluated by DCFH-DA assay. Oxidation of DCFH-DA to highly fluorescent compound DCF by H_2O_2 was inhibited by the scavenging activities of EGCG. Error bars show the standard deviation.

3.2. Antioxidant activity of the spray-dried pure EGCG microparticles

It has been reported that the antioxidant activity of EGCG decreases with increasing temperature and prolonged exposure to air (Su et al., 2003). In the current study, a spray drying process often took 2–3 h to complete, during which the feed solution was continuously exposed to air flush. To determine the loss of antioxidant activity due to exposure to air, the EGCG microparticles collected at the first hour and the second hour of spray drying from the big drier were assayed for their antioxidant activity using DCFH-DA assay. DCFH-DA converted to the highly fluorescent compound DCF (2′,7′-dichlorfluorescin) when oxidized by H_2O_2. The conversion to DCF can be inhibited by potent EGCG, in which H_2O_2 is scavenging by EGCG. In the DCFH-DA assay, the fluorescence intensity of DCF of different samples (containing EGCG, H_2O_2 and DCFH-DA mixture) was measured. A lower fluorescence reading indicates a higher antioxidant activity of EGCG.

The results of DCFH-DA assay are shown in Fig. 6. Regardless of H_2O_2 concentration, positive and negative controls gave the lowest and the highest fluorescence readings, respectively, indicating that untreated EGCG has the highest antioxidant activity while oven dried EGCG has the lowest antioxidant activities. Fig. 6 shows that spray dried EGCG obtained from big drier lost some antioxidant activities compared to untreated EGCG as expected. The overall activity retained in spray dried EGCG is still higher than that of the negative control, indicated that this spray drying process can preserve EGCG potency better than traditional oven dry process. The level of antioxidant activity of samples collected at different time point are similar, indicating that the additional 1 h exposure to air flush at room temperature did induce some loss of antioxidant activity, but the effect was little compared to that induced by oven drying.

The results of DCFH-DA assay of EGCG particles obtained from mini drier at two drying temperatures are shown in Fig. 7. As expected, EGCG particle lost some antioxidant activity after being spray dried, but the overall antioxidant activity is higher than that of oven dried EGCG. Although the use of drying temperatures of 95 °C and 105 °C led to different particle morphologies (Fig. 4), the antioxidant activities of these particles appeared similar as revealed from Fig. 7.

DPPH assay was employed to determine EC50 of EGCG treated with different condition. Radical scavenging activity of EGCG was evaluated at various concentrations as showed in Fig. 8. Oven dried EGCG (Negative control) had the highest EC50 value of 3.477 μM to scavenge 30 μM of DPPH, indicating that its antioxidant activity

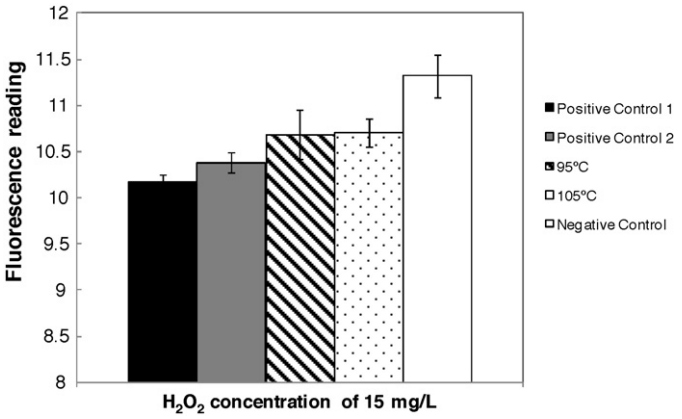

Fig. 7. H_2O_2 scavenging activities of spray dried EGCG obtained using the mini drier at different temperature were evaluated by DCFH-DA assay. Error bars show the standard deviation.

was the lowest amongst all the samples tested. The EC50 value of untreated EGCG (positive Control 1) is 3.028 μM. The EC50 value of residual EGCG remained in reservoir after spray drying (positive Control 2) is 3.056 μM. The EC50 value of spray dried EGCG obtained at 95 °C is 3.075 μM. The EC50 value of EGCG particles dried at 105 °C is 3.029 μM.

These results agree with the results obtained using DCFH-DA assay, suggesting that during spray drying, the exposure of feed solution to air flush before atomisation resulted in some loss of antioxidant activity; after the feed solution was sprayed into the drier, the heating might contribute to further activity loss. Nevertheless, the overall loss of antioxidant activity was little; the spray dry process can produce EGCG particle with higher antioxidant activity compare to oven dry process.

3.3. NMR analysis of the spray-dried pure EGCG microparticles

Spray dried EGCG samples were subjected to ^{13}C and ^{1}H analysis using liquid state NMR to examine for potential structural change in EGCG during spray drying. Representative ^{1}H NMR spectrum of EGCG obtained from mini drier at 105 °C is shown in Fig. 9b. Fig. 9a shows the ^{1}H NMR spectrum of untreated EGCG for comparison. The assignment of the proton resonances of EGCG in Fig. 9a is consistent with the data reported by Peres et al. (2010) and Inoue et al. (2002). The ^{1}H NMR (D_2O, δ ppm) shows that the signals of 2.8-3 are assign to C-ring CH_2, 5.1 and 5.6 to C-ring CH, 6–6.1 to A-ring CH, 6.5 to B-ring CH, and 6.9 to D-ring CH. Fig. 10a and b shows the ^{13}C NMR spectral of untreated EGCG and spray dried EGCG obtained from mini drier at 105 °C, respectively.

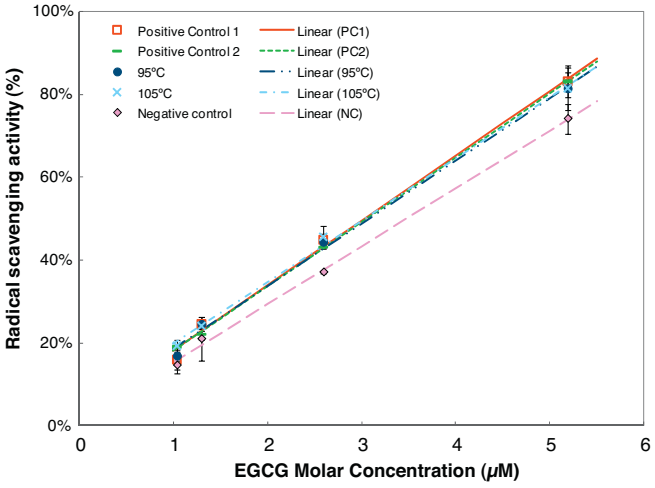

Fig. 8. Radical scavenging activities of EGCG microparticles obtained from mini drier at different temperature were evaluated by DPPH assay. Error bars show the standard deviation.

Both ^{13}C and ^{1}H spectrum of spray dried EGCG appeared to be similar to that of untreated EGCG. The peaks did not show any significant shift or variance in value after being spray dried, indicating that the spray-dried EGCG retains its original chemical structure. There was a small peak observed at approximately 1.85 ppm in the ^{1}H spectrum of 105 °C-dried EGCG, which was not observed for the original EGCG. This peak was probably cause by residual contaminant such as acetone in the NMR tube. It has been reported that at high temperatures, EGCG could epimerize to GCG (Gallocatechin Gallate) at C-2 position (Ikeda et al., 2003; Seto et al., 1997). As shown in Fig. 10a and b, no shift in δ 77.1 (C-2) peak was observed. Hou et al. (2005) reported that EGCG underwent auto-oxidation to form dimer under cell culture conditions, this oxidative product exhibits lower tumour inhibition activity. No oxidative product of EGCG was observed in the ^{13}C and ^{1}H spectrums. In conjunction with the results given by the DCFH-DA and DPPH assays, it can be said that EGCG microparticles reported in this study retained most of its antioxidant activity after spray drying.

3.4. The effect of lactose as coating

Lactose is a common excipient used in pharmaceutical industry and its effect on drug release is well studied (Chow et al., 2007; Gao et al., 1996; Talukdar and Kinget, 1995). Lactose has been studied for its potential to act as a thermoprotective agent during convective drying (Santivarangkna et al., 2006). Lactose is chosen as a model coating material in this study, to investigate whether it can be protect EGCG activity in spray drying.

EGCG and lactose were mixed at an empirical ratio of 1:4 and subjected to spray drying using the big drier. Five different inlet temperatures (70 °C, 80 °C, 90 °C, 110 °C and 13/0 °C) were used, and the DCFH-DA results of EGCG activity at these five conditions are shown in Fig. 11. Similar to the results of pure EGCG microparticles, the lactose-added EGCG microparticles showed a lower antioxidant activity than the positive control, but a higher antioxidant activity than the negative control. The antioxidant activities of EGCG particles obtained under five inlet temperatures were of similar level, indicating that the coating material can reduce the detrimental effect cause by increasing temperature on EGCG.

4. Discussion

4.1. Monodisperse droplet drying technique for the production of bioactive microparticles

It is well known that monodisperse particles offer various practical advantages compare to conventional particles with similar average sizes but a broader size distribution, the use of monodisperse particles enable controlled drug release, and the injection of large particles as particles with large size distribution increases the likelihood of needle clogging (Shutava et al., 2009; Xu et al., 2009). It is desire to find an economical and efficient process to produce monodisperse particles in large scale.

The ability to obtain droplets with narrow size distribution is the prerequisite to achieve precise control of drying history that each particle experience and to control the resulting moisture content, particularly for the drying of heat-and oxygen-sensitive material. When drying antioxidant, if each antioxidant particle undergoes a different drying history in spray drier, large variation in moisture content will be found in products, which consequent to large vari-

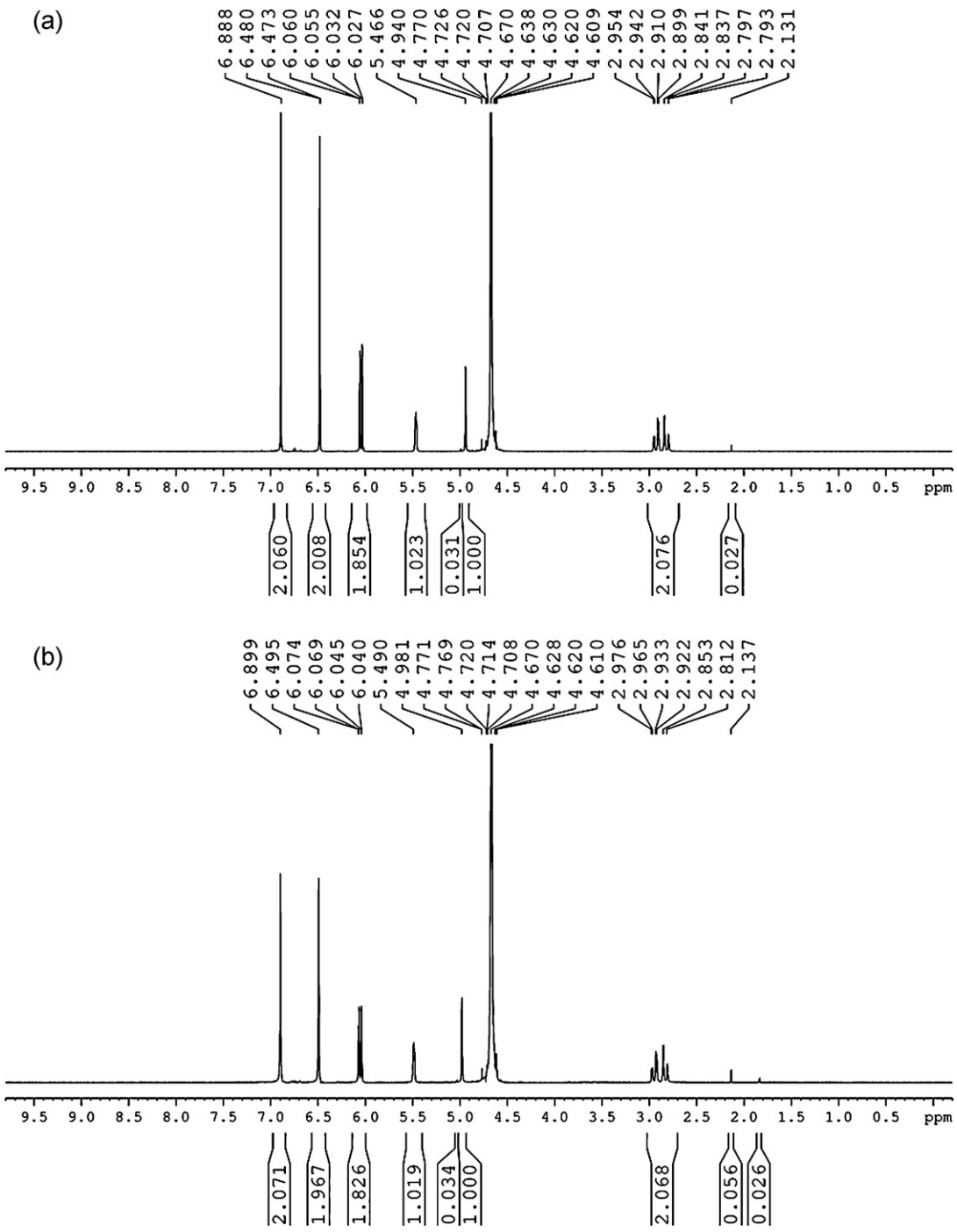

Fig. 9. Proton NMR spectrum of EGCG, obtained with liquid state NMR using D_2O as carrier. (a) Original EGCG, and (b) 105 °C spray-dried EGCG in mini drier.

ation in antioxidant activity as the denaturation of such materials is usually a function of temperature and moisture content.

The monodisperse spray drying technique described here is one of the methods that allow EGCG particles with high antioxidant activity to be produced. Compared to other formulation methods such as emulsification, homogeneous precipitation and template-assisted method, the current spray drying technique does not require purification step to remove solvents and template, it enables rapid and single-step production at large scale, and is therefore more economical. When compared to traditional freeze-drying process that offers high level of EGCG activity retention but involve the use of refrigeration, spray drying is more cost effective. Traditional spray drying technique generates droplets with large size distribution (Patel and Chen, 2007; Wu et al., 2007), thus it is unable to guarantee similar drying history for individual particles. Also, when compared to conventional spray drying, the current method integrates an atomisation procedure, which allows the production of monodisperse microparticles. Although spray dried particles with narrow size distribution have been reported from other spray drying approaches, however, the narrow size distribution was often achieved with the use of a specially designed cyclone for particles separation, where only a fraction of the spray dried particles were used as final product. The current method is therefore more cost effective in producing EGCG microparticles with high activity retention. The droplets generate from the atomiser undergo similar drying histories inside the drier, and give rise to particles with

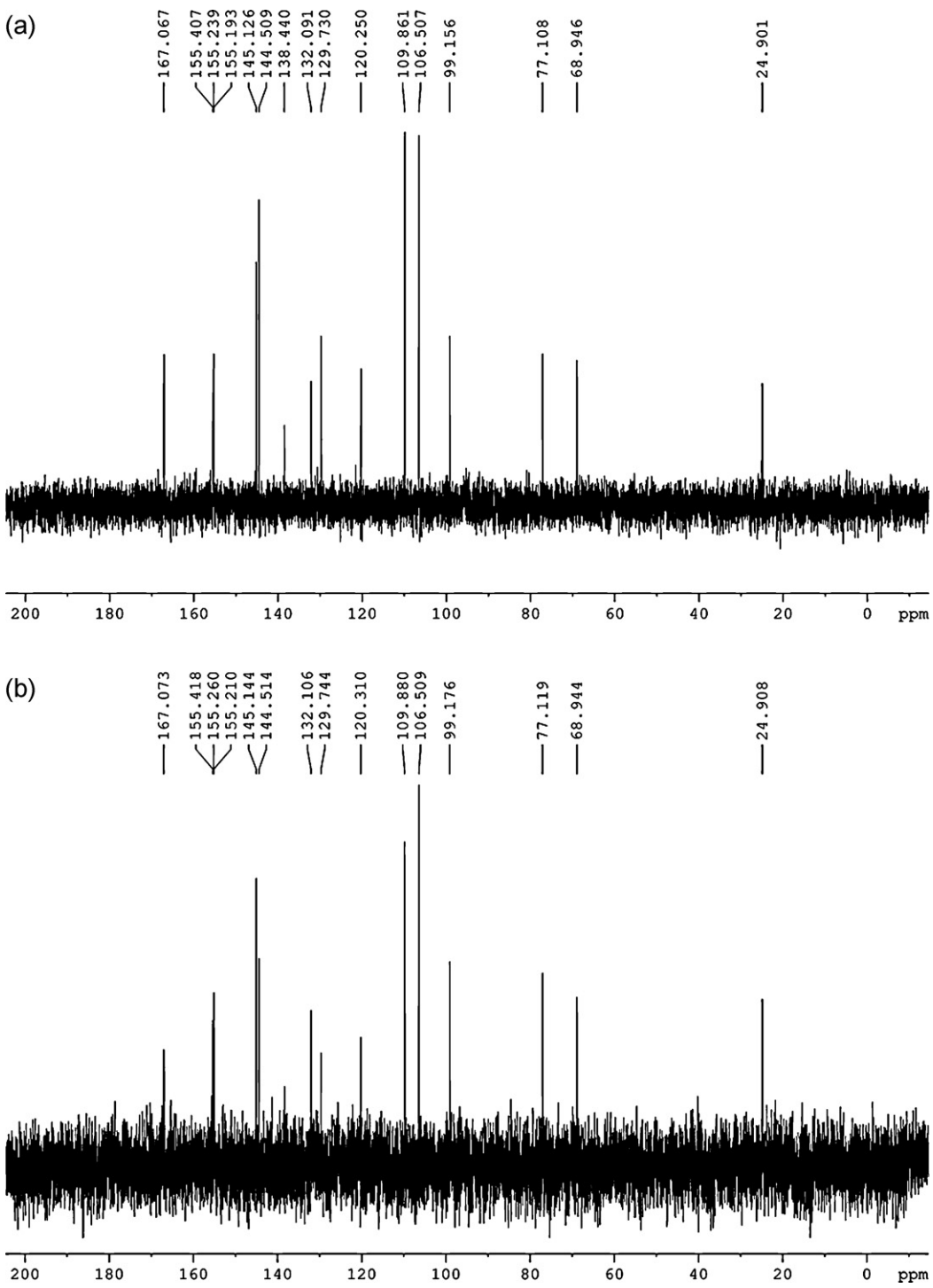

Fig. 10. Carbon NMR spectrum of EGCG, obtained with liquid state NMR using D_2O as carrier. (a) Original EGCG, and (b) 105 °C spray-dried EGCG in mini drier.

similar bioactivities. This method substantially improves the overall quality of such bioactive powders. Future studies can be focused on studying the release and bioavailability of such EGCG microparticles when different excipients are used, thus realizing an efficient production process for improved EGCG delivery.

4.2. The effect of temperature on particle size and morphology of EGCG

In conventional spray drying, the droplet size is largely affected by the orifice size. Both of mini and big drier were equipped with an atomiszer. It is known that the use of ultrasonic energy can enable generation of smaller droplets with high sphericity and uniform size distribution (Rajan and Pandit, 2001). A study (Rajan and Pandit, 2001) proposed the empirical correlation below to predict the droplet size generated from atomisation:

$$d_p = \text{constant} f^{-0.66} Q^{0.207} \sigma^{0.11} \rho^{-0.274} \eta^{0.166} \left(\frac{\text{power}}{\text{area}}\right)^{-0.4}$$

This correlation suggests that droplet size d_p can be decreased by increasing the vibration frequency f, decreasing the liquid flow rate Q, or by manipulating the physio-chemical properties (e.g., den-

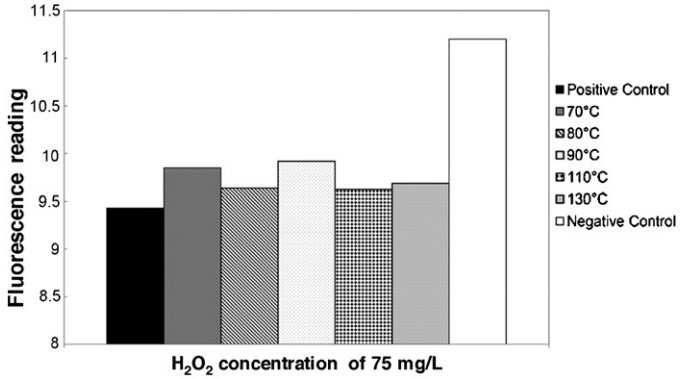

Fig. 11. H_2O_2 scavenging activities of lactose-added EGCG microparticles were evaluated by DCFH-DA assay, 5% (w/v) lactose-added EGCG spray dried in big drier, with an EGCG:lactose ratio at 1:4.

sity ρ, surface tension σ, viscosity η) of liquid. Despite the nozzles (orifice: 50 μm) used in both mini drier and big drier having the same size, the particles obtained from the two driers are of different size due to the difference in size of droplet being generated by the atomiser. Spray dried particles obtained from mini drier are smaller than that from big drier, due to the higher vibration frequency and lower mass flow rate being employed. Another advantage of using atomisers is that the spray contains droplet with little momentum (Rajan and Pandit, 2001), therefore shear stress induced on EGCG is also minimal. The resulting particles can therefore retain high antioxidant activities.

The particles obtained from mini drier at 95 °C has uniform size and morphology, this suggested that the atomised droplet experience similar evaporation process in the drying tower. The particle morphology is known to be governed by various factors including diffusion rate, evaporation rate, liquid physio-chemical properties and capillary forces resulted from water removal during drying (Yao et al., 2008; Hassan and Mumford, 1996). Yao et al. (2008) proposed that particle morphology can be predicted by the ratio of shrinking velocity of droplet surface multiple by droplet radius to the internal diffusion coefficient of droplet. The convex-concave shape particles obtained in our study can be explained by the slow diffusion rate of EGCG molecule inside the droplet. As water is removing from the droplet surface, an increase in EGCG concentration near the surface occurs, and results in an EGCG solid crust being formed on the droplet surface. The thickness of the crust continue to increase until a hollow particle is form, the crust eventually collapse into a convex-concave shape particle.

For EGCG droplet being dried by the big drier at 130 °C, there is an increase in EGCG diffusion rate inside the droplet due to the higher temperature used. The EGCG molecules are able to diffuse into the core of the droplet. The droplet forms a porous-like crust as water is being removed. The crust eventually shrinks to forms corrugated surface. It is note that diffusion rate inside the droplet is not even, this can be cause by the temperature gradient in the drying tower, where the top side of the droplet is expose to higher temperature and the bottom side is expose to lower temperature. This temperature gradient results in a higher diffusion rate at the top of the droplet, which allows EGCG molecules to re-homogenize quickly as water is being removed and form less porous-like structure. The overall result is the formation of a plano-convex shape particle with one flat side and one corrugated side.

It is interesting to note that EGCG particles obtain from mini drier at 105 °C exhibited two distinctly different morphologies, one of the population has biconcave morphology similar to that obtained at 95 °C which can be explained by the slow diffusion rate inside the droplet. The second population exhibit spherical morphology with corrugated surface, which can be explained by the higher diffusion rate inside the droplet. Despite drying in the same batch, droplets spreading to different region of the drying tower may experience slightly different temperature; the droplets therefore experience slightly different evaporation process and may lead to formation of particles with different morphology. This observation indicated a threshold for the formation of porous shape particle at a temperature of ~105 °C, and concave shape particles form below the threshold.

The antioxidant activity of EGCG microparticles were analysed by DCFH-DA and DPPH assays as shown in Figs. 7 and 8, the particles obtained at 95 °C and 105 °C with different size and shapes showed very little difference in antioxidant activities. It has been reported that the shape of inorganic nanoparticles affects their functionalities such as cellular uptake, catalytic activity and antibacterial activity (Huang et al., 2010; Narayanan and El-Sayed, 2004; Pal et al., 2007). Similar report, however, has not been found for organic microparticles.

4.3. Protective mechanism

The high activity retention is consider to be due to two major reasons: (1) low inlet temperature in our driers compare to typical spray drying process that heat biopharmaceutics to 130–140 °C (Ameri and Maa, 2006), and (2) evaporative cooling that counteracts the effect of heat convection. When feed solution is being atomised into the drying tower, the rise in temperature promote water evaporation, the heat loss from evaporation significantly reduce the wet-bulb temperature of the droplet; the wet-bulb temperature limits thermal inactivation of EGCG (Lievense and van't Riet, 1993).

It is reported that the wet-bulb temperature in spray dryer remains below 40 °C as long as the temperature of the drying air does not exceed 150 °C (Lievense and van't Riet, 1993), therefore the EGCG droplet/particles might be maintained at a temperature lower than 40 °C for the duration of drying. Fig. 11 shows that varying the inlet temperatures from 70–130 °C has little effect on the final activities of EGCG microparticles, as the drying temperatures of 60–130 °C will lead to wet-bulb temperatures ranging from 30 °C to 45 °C. This temperature range has been reported to have little impact on the antioxidant activity of EGCG (Su et al., 2003). In addition, lactose might offer further protection on EGCG antioxidant activity above 60 °C. These results suggest that during spray drying, the effect of excessive heat on the EGCG antioxidant activity was minimized. We therefore believe that the present experimental design is suitable for EGCG drying, and can facilitate production of monodisperse EGCG microparticles with high antioxidant activity.

5. Conclusion

This study investigated the use of low temperature spray drying systems to produce EGCG microparticles. We had successfully generated EGCG antioxidant microparticles with monodisperse size. These monodisperse EGCG particles experienced similar drying histories and had well-controlled qualities. It was found that most of the antioxidant activities were maintained in EGCG microparticles being spray dried at temperature ranging from 95 °C to 130 °C. This study also demonstrated the feasibility to produce monodisperse EGCG with coating in a one-step process using our spray drying system. Results showed that lactose coating is able protect EGCG from heat deterioration. This study demonstrated that spray drying is a viable and rapid approach to produce antioxidant microparticles that retain high potency. Further optimization of the drier system can permit the production of particles with smaller sizes or even at nano scale. This monodisperse spray drying technique shows great potential as an efficient and economic process for the

production of heat and oxygen sensitive microparticles for drug delivery.

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