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Research Article

Effects of Spray Drying Conditions on the Physicochemical and Antioxidant Properties of the Licorice (*Glycyrrhizaglabra*) Powder and Evaluation of their Antimicrobial Activity

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ABSTRACT

The effects of varying maltodextrin concentrations (10%, 20% and 30%) and spray drying temperatures (120, 140, 160, 180 and 200 °C) on the physicochemical, antioxidant properties and antimicrobial activity of licorice powder were studied. These include moisture content (MC), bulk density, pH, water activity (a_w), water solubility index (WSI), hygroscopicity, color characteristics, total carotenoid content (TCC), encapsulation efficiency (EE), total phenolic content (TPC) and total antioxidant activity (TAA). Moreover, the inhibitory effect of licorice products as antimicrobial agents was investigated. From the observations, there was hardy powder accumulated in the collector when maltodextrin was not added in the feed. The particles produced were very sticky and mainly deposited onto the wall of drying chamber and cyclone and could not be recovered. In was observed that with the addition of maltodextrin, the condition was improved. Addition of 20% maltodextrin to the feed appeared to give better results than addition of 10% maltodextrin. These results showed that maltodextrin was a useful drying aid in spray drying of licorice aqueous solutions and improved its yield. Moisture content, bulk density, hygroscopicity, color characteristics, total carotenoid content (TCC), encapsulation efficiency (EE) and total antioxidant activity (TAA) of licorice powder were significantly affected by maltodextrin concentration and the inlet air temperatures. However, pH, a_w and water solubility index (WSI) were not significantly influenced by the spray drying conditions. The increase in drying temperature and maltodextrin concentration decreased the total antioxidant activity (TAA) of the licorice powder. Morphological study revealed that at higher inlet temperatures the spray dried powder had small sized particles that were densely packed. Spray dried licorice powder made with 20% maltodextrin and processed at 160 °C inlet temperature had less hygroscopicity, a_w, a good quality licorice powder, acceptable color, TCC and TAA. The results obtained triggered to the effect of licorice products as antimicrobial agents. It was observed that the inhibitory effect of licorice increased by increasing its concentrations. It could be also noticed that the antimicrobial activity of reconstituted licorice powder was the highest among its ethanolic or water extracts.

Key words: Licorice, Liquorice, Spray drying, Maltodextrin, Antioxidant properties, Antimicrobial activity, Encapsulation.

INTRODUCTION

The licorice (liquorice) plant has a long and storied history of use in both Eastern and Western cultures pre-dating the Babylonian and Egyptian empires [35]. The genus name *Glycyrrhiza*is derived from the ancient Greek word for 'sweet root' (Gr. *Glykos* (sweet) + *rhiza* (root)), which was later Latinized to *liquiritia*and eventually to licorice [39].

The ancient Greeks and Romans are known to have such cultivated plant in the third century. Licorice grows wild in Asia and Europe and is extensively cultivated in China, Russia, Spain, Persia, and India.Licorice (*Glycyrrhizaglabra)* roots and rhizomes are extensively used in herbal medicines for their emollient,anti-inflammatory, anti-microbial,anti-viral, anti-allergic, anti-oxidant, gastro-protective, and anti-cancerous properties. It is widely used worldwide in food, confectionery and pharmaceutical products, such as cough syrups, herbal supplements, chewing gums, drinks, and candy. It is a powerful natural sweetener, 50–170 times sweeter than sucrose. The chemical constituents of the roots include several bioactive compounds, such as glycyrrhizin (~16%), different sugars (up to 18%) flavonoids, saponoids, sterols, starches, amino acids, gums and essential oils.

Kitagawa (2002) reported the detailed structures of 33 constituents in licorice roots and their sweetness. Glycyrrhizin is a water-soluble pentacyclictriterpenoid glycoside responsible for the sweetness of licorice and its aglycone is responsible for various medicinal attributes and clinical applications in the treatment of spleen, sore throat, bronchitis, liver, kidney, and ulcer. The glycoside usually occurs in a combined calcium or potassium

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salt formof glycyrrhizic acid (GA) which is a weak acid containing three carboxyl and five hydroxyl groups. The acid form of glycyrrhizic acid is not particularly water-soluble, but its ammonium salt is soluble in water at pH greater than 4.5. The mono-ammonium salt of GA is used as an anti-inflammatory and anti-allergic remedy for the treatment of bronchial asthma, eczemas and other diseases.

Licorice has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison’s disease and rheumatoid arthritis and has been used as a laxative and expectorant [4,52]. Among its most consistent uses are as a demulcent for the digestive system, totreat coughs, to soothe sore throats, and as a flavoring agent. The tobacco industry is the primary user of licorice derivatives in the United States, with the remainder equally divided among the food and pharmaceutical industries.

As with most plant extracts, the number of chemical constituents is potentially vast and greatly influenced by a constellation of genetic, environmental, and processing factors; licorice root extract is no exception. A detailed examination of the components identified in licorice root extract is beyond the scope of this assessment, but has been reviewed by other authors [15,51]. The fresh root contains about 20% of water-soluble extractives, and much of this—typically 3–5% of the root—is composed of glycyrrhizin, present as a mixture of potassium and calcium salts. The bright yellow color of licorice root is provided by flavonoids, particularly liquiritin, isoliquiritin and their corresponding aglycones, which typically comprise 1–1.5% of the water soluble extract. Licorice extract also contains reducing and nonreducing sugars, starch, plant gums, resins, essential oils, inorganic salts and low levels of nitrogenous constituents such as proteins, individual amino acids, and nucleic acids [54].

Methods of preparation of glycyrrhizic acid (GA) from licorice roots were investigated by different researchers.Most accepted technologies of the extraction of GA from the roots include extraction with hot water at ambient pressure in the presence of various additives like alkalis, mineral acids, ethyl alcohol, etc., such as, aqueous ammonia [31], methanol, and ethanol [25]. The primary aqueous extract of licorice roots contains GA and many other water-soluble substances and is subjected to further processing for more purified products [53]. It is desirable to have it in a powder form for ease to consume its nutrients and the attractive color.

Spray drying has been widely utilized for commercial production of driedfruits, vegetables and natural drinks. Spray-dried powders have good reconstitutional characteristics, low water activity and are suitable for transportation and storage. Furthermore, it is a highly appropriate process for heat sensitive components such as carotenoids. Spray drying has been successfully applied for carotenoid stability in plant foods such as carrots, tomato pulp, and sweet potato [19,21,28].

Several additives such as maltodextrin, gum Arabic and gelatin may serve as drying aid to facilitate drying. Currently, maltodextrin is one of the common drying aids for spray drying owing to its beneficial role as a carrier or an encapsulating agent in increasing the stability of carotenoids, reasonably cheap and commercially available. The addition of maltodextrin before spray drying has been reported to be effective in preserving carotenoids such as β-carotene [12]; carrot carotenes [49]; guava juice [10] and pineapple juice [1]. Furthermore, colour of foods is one of the most important sensory attributes which is affected by many factors during spray drying such as the inlet temperature and additives [1]. However, little information is published on preserving ofLicorice drinks and no study on spray drying conditions using maltodextrin as the carrier/encapsulating agent has been reported for producing Licorice powder.

This paper reports the effects of varyingmaltodextrin concentrations and spray drying temperatures on the physicochemical and antioxidant properties of licorice powder. These include moisture content, bulk density, pH, water activity, water solubility, hygroscopicity, colour characteristics, carotenoid content, encapsulation efficiency, total phenolic content and antioxidant activity. Inhibitory effects of the licorice powder and extracts (water and ethanolic) on the microbial activity for some microorganismswere also aimed.

Materials and Methods

1. Chemicals:

All chemicals used in this research, being n-Hexane 95%, acetone ≥ 99.5%, carotene (approx. 2**:**1 of **β:α**) mixed isomers from carrots, ≥95% (HPLC) powder form, sodium bisulfate ≥99%, potassium persulfate 99.99% metal basis, methanol spectrophotometric grade, trolox ((S)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid, 98%), and ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium) were purchased from Sigma–Aldrich (Louis, USA), andaqueous NH_3 (25%) from Merck.Maltodextrin DE**10** (MD) was purchased fromGlucidex®, (Roquette, France).

2. Microorganisms:

Sixmicrobial strains, namely: *Escherichia coli* O157:H7;*Bacillus cereus; Salmonella typhimurium*; *Staphylococcus aureus*; *Aspergillusniger and Saccharomyces serveciae* were obtained from Microbiological Resources Centre (Cairo Mircen), Faculty of Agriculture, Ain Shams University, Egypt, 2013. These Microorganisms were checked for purity and identity and always generated to obtain active microorganisms. The cultures were stored in a refrigerator at 4 ± 1 ºC and they were reactivated monthly on the suitable media.

3. Licorice (or Liquorice) root preparation:

3.1. Licorice root (Glycyrrhizaglabra)products preparation:

Licorice root was purchased from a local market in Cairo, Egypt (Produced in eastern Syria, licorice root was prepared in Ramzi manufactory as ‘‘Ramzi Licorice root products’’). For each experimental run, the licorice root (0.5 kg) was extracted by water with ammonia (0.01% w/v) (2.5 L), in the ratio of 1–5, at 50°C temperature for 2.5 h. After filtration the residue was extracted twice with 2.5 L of water with ammonia (0.01% w/v) under the same condition for 2.5 h. The extracts were combined. The resulting licorice extracts were twice filtered using a filter screen of 100 µm mesh to avoid blocking of the dryer atomiser. The licorice extracts were stored in a refrigerator at 4 ± 1°C.

Next, different ratios of commercial maltodextrin (10 DE)were added into the licorice water extracts, which were blended and finally filtered before spray drying. The three ratios of maltodextrin(10 DE) to the licorice water extracts were 10%, 20% and 30% weight/volume (w/v). The ratios chosen were deduced from literature and other runs with air drying. Preliminary spray drying trials showed that when the concentration of maltodextrin(10 DE) was lower than 10%, there was pumping problem and most materials stuck on chamber wall; when the concentration of maltodextrin was higher than 30%, the resulting powder lost its attractive color. Feed material for all the runs had to come from one master batch to be consistent, so there was just enough for duplicated runs.

3.2. Spray drying conditions for licoriceaqueous solution:

The feed mixtures comprising added maltodextrin and licorice aqueous solutions were spray-dried in a Lab Plant BÜCHI Mini spray dryer B-290 (Lab Plant Ltd., Switzerland). The inlet temperatures/measured outlet temperatures were 120°C/83°C, 140°C/94°C, 160°C/103°C, 180°C/112°C and 200°C/125°C. The drying air flow rate, compressor air pressure and feed rate were constant, at 56 ± 2 (m^3/h), 0.05MPa gauge and 12–14 ml/min, respectively. After the spraying process, the licorice powder was collected in a glass collection vessel wrapped with aluminium foil, and immediately stored in a dessicator containing silica gel for equilibration to room temperature. The spray-drying processes were all carried out in duplicate.

4. Analytical methods:

All analytical measurements were carried out in triplicate.

4.1. Moisture content:

The moisture content of licorice samples was determined by drying at the temperature of 105 °C in the oven until a constant weight was obtained.

4.2.Water activity (a_w):

A water activity meter (AquaLab PawKit, Decagon Devices, USA) was used to measure a_w of the spray-dried licorice powders.

4.3. pH determination:

The pH value of licorice powders was determined by blending 5g powder with 25mldeionized water at20°C, using thepH meter calibrated with standard bufferspH 7 and 4.

4.4. Water solubility index (WSI):

The WSI of the licorice powders was determined using the method described by Mukhopadhyay and Panja [31]. Spray-dried licorice powder (2.5 g) and distilled water (30 ml) were vigorously mixed in a 100 ml centrifuge tube, incubated in a 37 °C water bath for 30 min and then centrifuged for 20 min at 10,000 rpm (11,410×g) in a Centrifuge (Beckman, USA). The supernatant was carefully collected in a pre-weighed beaker and oven dried at a temperature of 105 °C. The WSI (%) was calculated as the percentage of dried supernatant with respect to the amount of the original 2.5 g licorice powder.

4.5. Bulk density:

Bulk density (g/ml) was determined by gently adding 2 g of licorice powder into an empty 10 ml graduated cylinder and holding the cylinder on a vortex vibrator for 1 min. The ratio of mass of the powder and the volume occupied in the cylinder determines the bulk density value [17].

4.6.Hygroscopicity:

For hygroscopicity, 1.5 g of the licorice powders was placed at 25 °C in an airtight container containing saturated solution of sodium carbonate. Sample was weighed after 1 week and hygroscopicity was expressed as gram of adsorbed moisture (**AM**) per 100 g of powder [6].

4.7. Color characteristics:

The color of licorice powder sample was determined using a Minolta Chroma Meter calibrated with a white standard tile. The results were expressed as Hunter color values of ***L*, a*,*** and ***b****, where ***L**** was used to denote lightness, ***a**** redness and greenness, and ***b**** yellowness and blueness. Prior to measurement, the powder samples were packed into a polyethylene pouch and measured. Hunter values of the samples for each treatment method were measured in triplicate.

Chroma, indicating color intensity, was calculated by the formula $(\boldsymbol{a}^{*2} + \boldsymbol{b}^{*2})^{1/2}$. The hue angle ($H$°) was calculated by the formula H° = arctan (***b*/a****). The hue angle values vary from 0° (pure red color), 90° (pure yellow color), 180° (pure green color) to 270° (pure blue color). The ratio of ***a*/b**** was also used for the color measurement. Total color difference or change between two samples was calculated by the formula as follows:

$$\Delta \boldsymbol{E} = \sqrt{(\boldsymbol{L}_0^* - \boldsymbol{L}^*)^2 + (\boldsymbol{a}_0^* - \boldsymbol{a}^*)^2 + (\boldsymbol{b}_0^* - \boldsymbol{b}^*)^2}$$

Where:$\boldsymbol{L}_0^*$, $\boldsymbol{a}_0^*$ and $\boldsymbol{b}_0^*$ are the values of the samples at zero time, and ***L*, a**** and ***b**** the measured values of each sample after processing or reconstitution [14].

4.8. Determination of total carotenoid content:

4.8.1. Extraction and separation:

A method described by Tran *et al.* [48] was employed, with some modifications, to extract the carotenoid content from the licorice samples. Approximately 0.1 g of licorice powder was weighted in beaker and then extracted with 10 ml of the solvent, which is a mixture of n-hexane**:**acetone (v/v 3**:**2). The residue was further extracted four times using a magnetic stirrer until colorless, each time with 5 ml of the solvent. The extracts were combined and washed twice to remove acetone, each time with 25 ml of distilled water in a separating funnel. A few drops of saturated NaClsolution were added to the funnel to facilitate phase separation. The upper layer was collected to measure total carotenoid content. The process was conducted under dim light and analyzed within one day.

4.8.2.Standard curve of carotene:

Carotene solution (0.0005–0.01 mg/ml) was used to construct the standard curve for the determination of total carotenoid content**(TCC)**. **The TCC** of **licorice** powders was spectrophotometrically determined at 473 nm and expressed based on carotene equivalents (mg/g of powder).

4.9. Encapsulation efficiency (EE):

A method described by Shu *et al.* [41] was employed, with some modifications, to calculate encapsulation efficiency. The **EE (%)** was determined as the ratio between the initial content of total carotenoids before spray drying and the content of the final powder product (mg/g powder).

4.10. Total phenolic content:

Estimation of total phenolic content was performed by Folin–Ciocalteu method described by Liu *et al.* [29] with some modifications. Briefly, 250 mg of sample were mixed with10 ml of 60 % acetone and the mixture was stirred for 30 min at 30°C. Then 60 µL of supernatant, 300 µL of Folin–Ciocalteau reagent and 750 µL of 20% sodium carbonate in water were added in 4.75 ml of water. The mixture was allowed to stand for 30 min. The absorbance was measured at 765 nm using double beam spectrophotometer.Results were calculated by a calibration curve obtained from chlorogenic acid and expressed as milligrams of chlorogenic acid equivalents (CAE) /100 g of dry weight.

4.11. Determination of total antioxidant activity by the ABTS assay:

The procedure for determination of total antioxidant activity followed the method of Thaipong *et al.* [46]. A 7.4 mM ABTS [2,2'-azino-bis (3-ethylbenzthiazonline-6-sulfonic acid)] solution and a 2.6 mM potassium persulfate solution were used as the stock solutions. The equal quantities of the stock solutions were mixed as the working solution and reacted for 12–16 h at room temperature in the dark. This solution was then diluted by mixing 1 mlABTS solution with 60 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Licorice sample extracts (0.15 ml) were reacted with 2.85 ml of the ABTS solution for 2 h in a dark situation. The absorbance was spectrophotometrically taken at 734 nm. The standard curve was linear between 0.025 and 0.8 mMTrolox. Results were expressed in mmoleTrolox equivalents **(TE)/g** of licorice powder.

5. Scanning electron micrograph:

Particle morphology was evaluated by scanning electronmicroscope ***(SEM)***. Powders were attached to a double sided adhesive tape on SEM stubs, coated withgold palladium under vacuum and examined with a JEOL scanning electron microscope (JXA-840 A, Japan, PN junction type, semiconducting detector). SEM was operated with 15 kV at magnification of 1000× and 5500×.

6. Antimicrobial activity of licorice products:

Six microorganisms *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Aspergillusniger and Saccharomyces serveciae* were tested. The nutrient agar medium for bacterial growth was used according to Difco-Mannual [13], while medium for Yeast and Mold growth was used according to Gagnon *et al.* [17].

6.1. Preparation of licorice water extracts:

Fifty gm ground licorice was soaked in 500 ml water for 48 h at room temperature with occasional shaking. Mixture was filtered through cheese cloth followed by filter paper. Water extract solution was freeze dried at –30 °C under high vacuum,the yield was highly hydroscopic powder.

6.1. Preparation of licorice ethanol extracts:

Fifty gm ground licorice was soaked in 500 ml 95% ethanol for 48 h at room temperature (25 ± 2 °C) with occasional shaking. Mixture was filtered through cheese cloth followed by filter paper. Ethanolic solution was evaporated with ethanol and yield was in the form of thick paste.

6.2. Measurement of inhibition zone (mm):

Paper-Disc plate technique was used to study the effect of licorice powder (reconstituted), and licorice (ethanolic and water) extracts on bacterial growth by measuring the diameter of the inhibition zone. A volume of 7 ml sterile assay agar was added to each of two Petri dishes and allowed to harden with slow shaking. Warmed agar (7 ml) inoculated with 0.5 ml of an active bacterial culture (containing 1×10^5 CFU/ml^{-1}) of the tested organism was added and allowed to harden in a refrigerator at 4±1 °C for 1 h, a disc paper (5 mm) was emerged in licorice extracts and placed on the top of the inoculated agar layer then dried at 25±5 °C for 30 min. Plates were kept at 4±1 °C for 1-2 h then incubated at 35±2 °C for 24-48 h and inhibition zones were accurately measured in millimeters (mm) [5].

6.3. Determination of minimum inhibitory concentration (MIC):

To determine the inhibitory concentration (MIC) of highly inhibitory licorice extracts, different amounts of selected powder (reconstituted), and(ethanolicand water) extracts from licorice were thoroughly mixed with nutrient agar medium to prepare 0.5, 1.0 and 1.5% concentration in medium. A drop of Tween 80 (1.12 v/v Tween/ethanol) was directly added to the licorice extracts before mixing with medium.

The melted sterilized medium with each concentration of licorice extract poured (15 ml) to a set of three dishes, after solidification the dishes were inoculated by streaking with an overnight suspension (broth culture) of test pathogenic microorganisms. The inoculated at 30±2 °C for 24-48 h and the lowest concentration of licorice powder (reconstituted), ethanolic and water extracts required to inhibit the growth of the test microorganism was designated as MIC[17].

7. Statistical analysis:

The experiments were carried out in duplicate and results were presented as mean values with standard deviations. Different mean values were analyzed by analysis of variance (ANOVA) and least significant difference (LSD). The graphs of mean values and error bar were created using Excel version 2003.

Results and Discussion

1. Effect of maltodextrin:

From the observations, there was hardy powder accumulated in the collector when maltodextrin was not added in the feed. The particles produced were very sticky and mainly deposited onto the wall of drying chamber and could not be recovered. Therefore, maltodextrin of 10% and 20% (of total feed solution) was added to the licorice aqueous solutions prior to spray drying to investigate its effect on the spray drying product. Maltodextrin (Glucidex®) used in this study was a low dextrose equivalent (DE) maltodextrin with DE of 10. Other researchers have reported that low DE maltodextrin had better nutrient binding properties [38]. It was observed that with the addition of maltodextrin, the condition was improved. Addition of 20% maltodextrin to the feed appeared to give better results than addition of 10% maltodextrin(Fig. 1A and B). These results showed that maltodextrin was a useful drying aid in spray drying of licorice aqueous solutions as it improved the yield of product.

2.Effects of spray drying conditions on the physicochemical properties of Licorice powder:

The effects of maltodextrin concentrations and different spray drying temperatures on the physicochemical properties of licorice powder are shown in Table 1. Results showed that increasing maltodextrin concentration and drying temperature resulted in a decreasing moisture content of the samples ($p < 0.001$). As maltodextrin concentration increased from 10% to 30%, the moisture content of samples significantly reduced from 4.94% to 4.10%. A similar trend was observed while increasing drying temperatures from 120 °C to 200 °C, resulting in a significant drop in moisture content from 5.32% to 3.98%.

For spray drying in general, increasing drying temperature resulted in greater loss of water of resultant powder, due to the higher rate of heat transfer into particles, causing faster water removal. This is also shown in our result, moisture content of powders reduced quickly as increasing air inlet temperature from 120 °C to 200 °C and maltodextrin concentration from 10% to 30%. Similarly, Goula *et al.* [20]; Chegini and Ghobadian [8]; Rodríguez-Hernández *et al.* [38] and Ersus and Yurdagel [16] reported that the moisture content of tomato powder, orange juice powder, cactus pear juice powder and black carrot powder decreased as drying temperature increased.

Moreover, in this study a decrease in the moisture content of licorice powder was also obtained when the maltodextrin concentration increased. Similarly, Abadio *et al.* [1] found that an increased concentration of maltodextrin(10 DE), from 10% to 15%, reduced the moisture content of resultant pineapple juice powders. A similar result was also reported by Grabowski *et al.* [21] who carried out tests on sweet potato puree powder. These findings could be explained by the fact that additional concentrations of maltodextrin resulted in an increase in feed solids and a reduction in total moisture for evaporation.

Fig. 1: (A and B) Spray drying with (A) 10% and (B) 20% maltodextrin.

Table 1: Physicochemical properties of spray-dried licoricepowders.

Particular		MC (%)	pH	a_w	WSI (%)	Bulk density (g/ml)	Hygroscopicity [g (AM)/100g]
Maltodextrin Concentration (MDC)	10%	4.94 ± 0.51^a	5.30 ± 0.11	0.52 ± 0.08	$93.46 + 2.03$	0.72 ± 0.11^a	$53.21 + 1.11^a$
	20%	4.45 ± 0.44^b	5.27 ± 0.16	0.45 ± 0.10	$94.33 + 1.15$	0.64 ± 0.09^b	$49.54 + 1.03^b$
	30%	4.10 ± 0.39^c	5.29 ± 0.18	0.42 ± 0.09	$93.11 + 1.91$	0.60 ± 0.10^c	$45.03 + 0.88^c$
Drying Temperature (DT)	120°C	5.32 ± 0.42^a	5.19 ± 0.10	0.54 ± 0.11	$94.98 + 1.87$	0.69 ± 0.10^a	$56.88 + 1.22^a$
	140°C	4.83 ± 0.37^b	5.32 ± 0.12	0.49 ± 0.10	$94.54 + 1.44$	0.64 ± 0.09^b	$49.74 + 1.72^b$
	160°C	4.44 ± 0.41^c	5.25 ± 0.14	0.45 ± 0.08	$94.36 + 1.13$	0.58 ± 0.08^c	$44.56 + 1.17^c$
	180°C	4.08 ± 0.22^d	5.29 ± 0.16	0.46 ± 0.11	$93.42 + 2.11$	0.57 ± 0.10^{cd}	$44.11 + 1.66^c$
	200°C	3.98 ± 0.29^d	5.37 ± 0.13	0.46 ± 0.12	$93.31 + 1.56$	0.54 ± 0.07^d	$41.60 + 1.32^d$
Significant Interaction		Significance					
MDC		***	NS	NS	NS	NS	***
DT		***	NS	NS	NS	**	**
MDC × DT		**	NS	NS	NS	NS	**

Values are mean ± SD (two replicates) after statistical analyses.
NS, *, ** and *** indicate not significant and significant at $p = 0.05$, 0.01 and 0.001, respectively.
The values in the same column followed by different superscripts (a–e) were significantly different ($p < 0.05$).
AM:adsorbed moisture.

The values of pH, a_w and WSI of the licorice powders in this study were not significantly affected by inlet air drying temperature and maltodextrin concentration ($p > 0.05$). For pH value, this finding is in agreement with the results of Gonzalez-Palomares *et al.* [18] who found that pH of the Roselle extract powders does not change with different air drying temperatures. Moreover, a_w is one of the most important factors that significantly influence the shelf life of the powder products. High water activity in products leads to shorter shelf life due to high free water for biochemical degradations. The deterioration of dried powder caused by microorganisms and biochemical reactions could be prevented at a_w lower than 0.6 [45]. The average a_w of powders in this study ranged from 0.42 to 0.54 (Table 1), and could be considered to be quite microbiologically stable. Additionally, from the results shown in Table 1, the a_w of powders decreased with increasing maltodextrin concentration. The results for a_w of the powders were consistent with the findings of the study carried out by Quek *et al.* [36]. They stated that the water activity of spray-dried watermelon powders was not significantly changed by inlet temperatures between 145 °C and 175 °C. Further, higher concentration of maltodextrin resulted in decrease in the a_w ofthe powders.

The WSI of samples in the study was not influenced by different drying conditions ($p > 0.05$). A similar observation was also reported by Sousa *et al.* [42] who studied spray-dried tomato powders. The range of WSI of samples in this study was 36.91–38.25%. These values were higher when compared to results for spray dried tomato powders, which ranged from 17.65% to 26.73% [42]. However, the licorice WSIvalues were much highercompared to those of pineapple juice powder, with an average value of 81.56% [1].

Further, the solubility of powders can be affected by many parameters such as initial compositions of the raw material to be spray-dried, the carrier agents, compressed air flow rates, and low feed rates [2,19]. For example, a superior water solubility property of spray-dried cashew apple juice powder was obtained by using cashew tree gum as the drying aid agent [11]. Therefore, further investigation may need to be carried out to identify methods for improving the water solubility of licorice powders further if desired.

In this study, the bulk density of licorice powders was significantly affected by the drying temperature ($p < 0.01$), with decreasing density observed with increased drying temperature. This is consistent with the findings of a number of studies, that increasing inlet air drying temperature results in reducing bulk density [9,20]. At very high temperatures, very high drying processes are achieved implying a lower shrinkage of the droplets, and so a lower density of the powder [26,50,8].

Maltodextrin level had significant ($p < 0.05$) effect on the hygroscopicity of the licorice powder. Hygroscopicity was lowest when 30% maltodextrin was used for encapsulation. Inlet temperature also influenced the hygroscopicity of the powder significantly. The highest hygroscopicity value of 56.88 g/100 g for licorice powder was obtained at 120 °C inlet temperature. When inlet temperature of processing was increased the hygroscopicity of licorice powder was decreased. Rodriguez-Hernández *et al.* [38]; and Cai and Corke [6] also observed a reduction in hygroscopicity with increasing maltodextrin concentrations in spray dried cactus pear juice powder andbetacyanin pigments, respectively. Maltodextrin is a material having the property of low hygroscopicity and its utility as acarrier material for spray drying [47] has beenestablished. Inlet temperature also influenced the hygroscopicity of the powder significantly. The highest hygroscopicity value of 56.88 g/100 g for licorice powder was obtained at 120°Cinlet temperature. When inlet temperature of processing was increased the hygroscopicity of licorice powder was decreased. The present findings are in agreement with Moreira *et al.* [30] but contradicts the findings of Goula *et al.* [20] and Tonon *et al.* [47] in their work on spray drying of tomato pulp and acai juice powder, respectively. Licorice powder showed greater tendency to adsorb moisture which may be due to the presence of higher level of carbohydrates in licorice root.

3. Effects of spray drying conditions on the color characteristics of Licorice powder:

Figs. 2 and 3 show the effects of different maltodextrin concentrations and drying temperatures on the color characteristics of spray-dried powders. In general, the color characteristics of spray-dried powders were significantly impacted by maltodextrin concentration and drying temperature. For the lightness, the color of products was significantly affected by maltodextrin concentration ($p < 0.01$). An increase in the lightness of products was significantly obtained by increasing maltodextrin concentration from 10% to 20%. However, there was no significant difference in lightness of samples when the concentration increased from 20% to 30%. A consistent result was also observed in terms of the color characteristics of the a^*/b^* value and the hue angle. The highest value of a^*/b^* and the lowest hue angle were obtained in the sample with an added 10% concentration of maltodextrin both indicating more yellowness. Moreover, the maltodextrin concentration also impacted on the chroma value of samples ($p < 0.001$). Decreasing the chroma from 32.16 ± 3.43 to 26.25 ± 2.11 was observed at increasing maltodextrin concentrations from 10% to 20%.

Spray drying temperature was another factor affecting the color characteristics of products, namely chroma, hue angle and a^*/b^* value, but not lightness. A significant effect of drying temperature on the a^*/b^* value and hue angle was statistically observed ($p < 0.01$). Loss of yellowness of samples, resulting in low a^*/b^* value and high hue angle, increased when increasing temperatures from 120 °C to 200 °C; however, no statistical difference in the value of a^*/b^* and hue angle between the temperatures of 120 °C, 140 °C and 160 °C, and no difference between these characteristics at 180 °C and 200 °C, was significantly observed. In contrast, the lightness of products was not significantly influenced by spray drying temperature ($p > 0.05$).

Furthermore, statistical interaction between maltodextrin concentration and drying temperature was significantly obtained in the lightness and chroma values of the powder products. For the a^*/b^* value and hue angle of products, however, there was no significant interaction between the two factors.

The total color difference (ΔE) of reconstituted licorice powders compared to feed mixtures before the spray-drying process is shown in Fig. 4. The ΔE of reconstituted licoricepowders was not impacted by maltodextrin concentration ($p > 0.05$); however, a significant effect of drying temperature on ΔE was statistically observed ($p < 0.001$). Increasing drying temperature significantly resulted in an increase in ΔE. Moreover, there was no significant interaction between maltodextrin concentration and drying temperature.

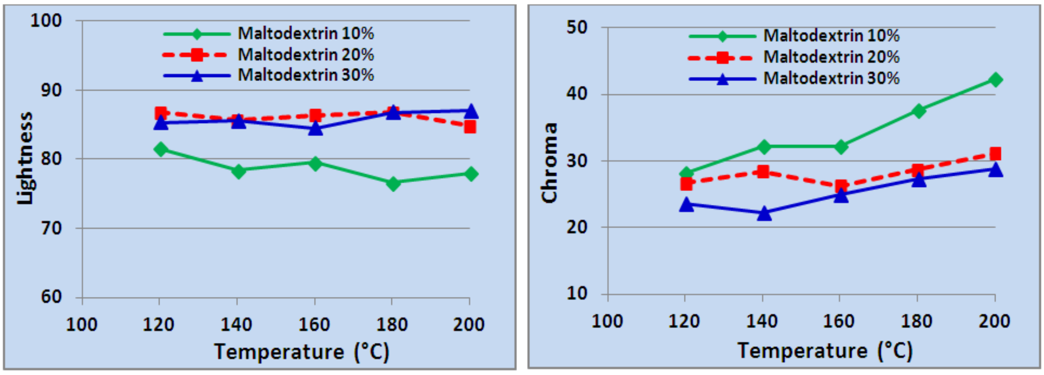

Fig. 2: The lightness and chroma of licoricepowders as a result of different spray drying conditions.

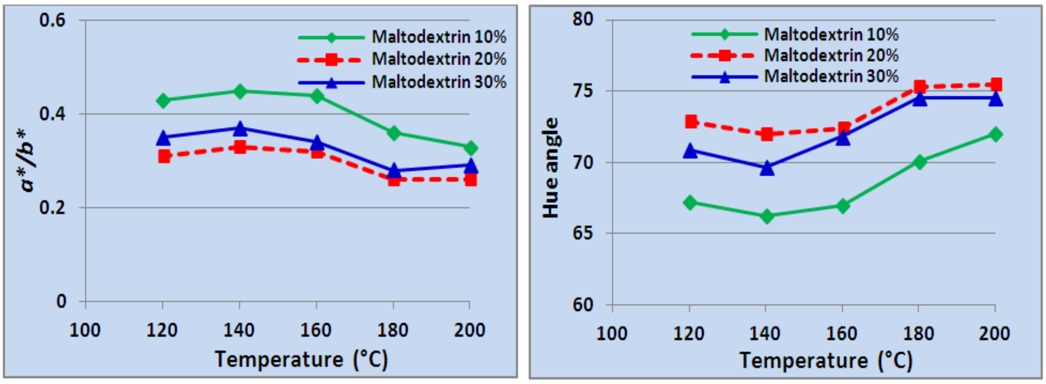

Fig. 3: The ratio a*/b* and hue angle of licorice powders as a result of different spray drying conditions.

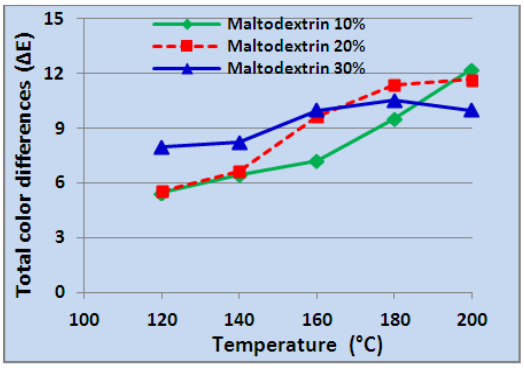

Fig. 4: Total color differences of reconstituted Licorice powders after spray drying process.

Generally, an increase in the lightness value of the powders was observed with an increased maltodextrin concentration. Because of white color of maltodextrin, a greater lightness of powders, represented by a higher *L** value,was obtained at higher concentrations of maltodextrin. Similar results were also found in spray-dried sweet potato powders [21] and in pineapple juice powders [1]. On the other hand, the lightness of licoricepowders in this study was not significantly influenced by the drying temperature. However, Sousa *et al.* [42] found that the highest value of lightness of spray-dried tomato powders was observed at the highest inlet drying temperature, indicating less darkness due to the pigment oxidation. In contrast, the lightness of watermelon powders reduced when inlet drying temperature increased due to thehigh content of sugar causing browning of powders [36].

The chroma value of licoricepowders was significantly affected by both the drying conditions of maltodextrin level and the inlet drying temperature. High chroma value of powders was observed at low maltodextrin concentration and at high temperatures. This could be due to significant interaction between the two investigated factors. This finding is in agreement with the results reported by Quek *et al.* [36].

Lower values of *a*/b** and higher hue angles were observed as a result of increasing maltodextrin concentration and increasing the inlet drying temperature. These results indicate that the loss of yellowness of powder products was increased in such spray drying conditions. A similar result was observed by Sousa *et al.* [42] who reported that a decrease in the value of *a*/b** in tomato powder was found with increasing the inlet drying temperature. Further studies confirmed that increased drying temperatures resulted in low retention in the redness color of tomato products [40]. The possible explanation for this phenomenon is that carrying out the spray-drying process with a high ratio of surface area and volume of feed mixture caused rapid pigment oxidation. Therefore, the spray drying conditions at high temperature resulted in a high loss of yellow color due to thermal degradation of carotenoid pigment. Goula and Adamopoulos [19] indicated that a higher loss of lycopene content in tomato powder was observed by increasing the air inlet temperature. In terms of the maltodextrin concentration, moreover, the lesser yellowness of licorice powders was due to the higher concentration of maltodextrin used in the spray-drying process. As previously mentioned by Grabowski *et al.* [21], increasing maltodextrin resulted in an increase in hue angle in sweet potato powders, indicating a loss of redness.

The trend for total color difference of reconstituted Licorice powder products as a result of the spray-drying process was similar to the results for yellowness in terms of being significantly affected by the inlet drying temperature; however, not by the maltodextrin concentration. The reduction of yellowness, indicated by high hue angle and low *a*/b** value, is the possible explanation for an increased total color difference in reconstituted Licorice powders due to high inlet temperature. Additionally, it can be clearly seen that total color differences(ΔE) is a function of value L^* a^* b^*, therefore, increase in lightness with increased inlet temperature was also a contribution to increasing ΔE. Contradictorily, Rodríguez-Hernández *et al.* [37]; and Grabowski *et al.* [21] indicated that the influence of maltodextrin concentration was found to be significant for the variation of ΔE in reconstituted cactus pear juice and sweet potato puree powders, respectively. Their different results might be due to different color characteristics of their raw materials and the different processing conditions.

4. Effect of spray drying conditions on the total carotenoid and encapsulation efficiency:

The total carotenoid content (TCC) and encapsulation efficiency (EE) of the licorice powder products as a result of different spray drying conditions are presented in Figs. 5 and 6, respectively.

The TCC in powder samples reduced from 1.92 mg/g to 0.66 mg/g of powder as the maltodextrin concentration increased from 10% to 30% ($p < 0.001$) at 160 °C. Further, the spray drying temperature also affected TCC; significant loss of TCC in samples was observed as temperature increased from 120 °C to 200 °C ($p < 0.001$). However, there was no statistical difference in TCC of samples at temperatures between 140 °C and 160 °C; between 160 °C and 180 °C; or between 180 °C and 200 °C. Overall the best TCC retained was found with using only 10 maltodextrin at 120 °C.

According to Goula and Adamopoulos [19], an increase in inlet drying temperature resulted in a greater loss of lycopene content in tomato powders. Similarly, Quek *et al*. [36] observed that a decrease in the lycopene and β-carotene content of spray dried water melon powder occurred as a result of increasing the inlet air temperature. The main reason for these findings was due to thermal degradation and oxidation. In addition to the inlet temperature, the loss of carotenoids in the licorice powder samples was also dependent on several factors, such as out let temperatures, droplet moisture content, oxygen and exposure to light. These factors are governed by processing conditions such as feed rate, initial feed solid concentration, drying and compressed air flow rate. Moreover, the higher moisture content was obtained by powder spray drying at lower inlet air temperatures. Increasing moisture content caused a higher loss of lycopene, however, when moisture content increased, a greater degree of aggregation occurred because of the natural stickiness of the product. This leads to there being lower oxygen exposure resulting in lower lycopene loss [19,20]. Moreover, carotenoids are easily vulnerable to thermal treatment and oxidative processes due to their structure which contains a conjugated double bond system over the entire length of the polyene chain [36].

The EE of the study samples was also significantly influenced by maltodextrin concentration and by drying temperature ($p < 0.001$). Increasing maltodextrin concentration resulted in higher EE; however, no difference in EE between the concentration of 20% and 30% was observed. Moreover, in general, EE of the samples reduced from 78.6% to 56.8 % as the drying temperature increased from 120 °C to 200 °C, respectively.

In terms of the effect of drying temperature, a similar pattern to the TCC loss as a result of increasing the inlet temperatures was also observed in relation to EE. This is indicated that increasing temperature resulted in reduction of EE. The explanation for this phenomenon is that the degradation of carotenoids at higher temperature, as discussed above, leads to reduce EE. Further, according to Shu *et al.* [41], the balance between the rate of water evaporation and film-formation may break due to high inlet temperature; therefore, wall system of microcapsules is broken down. This phenomenon will cause a low EE. Similar findings werealso reported by authors [41,44]. Overall, Fig. 4

shows that it was not required to go past 20% maltodextrin for higher EE.

In this study, the TCC was significantly decreased when maltodextrin concentration was increased from 10% to 30%. This is due to high maltodextrin concentration leading to lower TCC obtained as the feed extract was constant. On the other hand, an increase in EE was observed as increasing maltodextrin concentration. This is well known that carotenoid content in powder is effectively protected at a high initial feed solid. Similar observations were found in other studies [38].

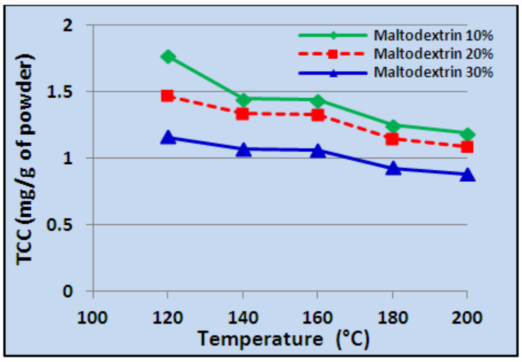

Fig. 5: Total carotenoid content (TCC) of spray-dried powders as a result of different drying conditions.

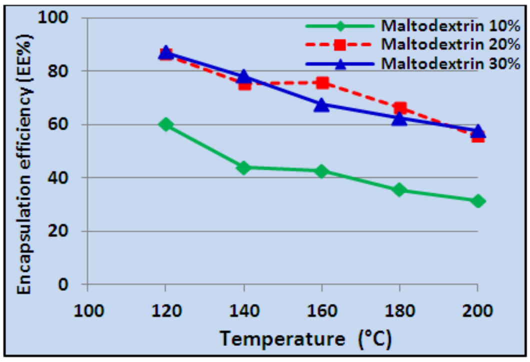

Fig. 6: Encapsulation efficiency (EE %) of spray-dried powders at different drying conditions.

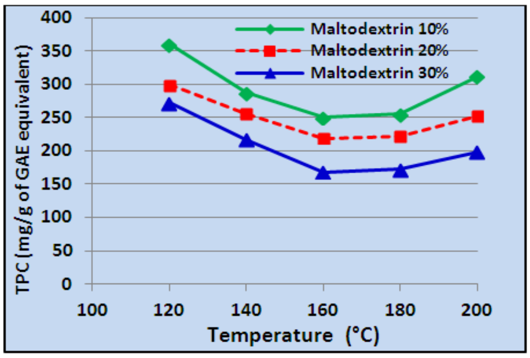

Fig. 7: Total phenolic content (TPC) of spray-dried powder under different dying conditions.

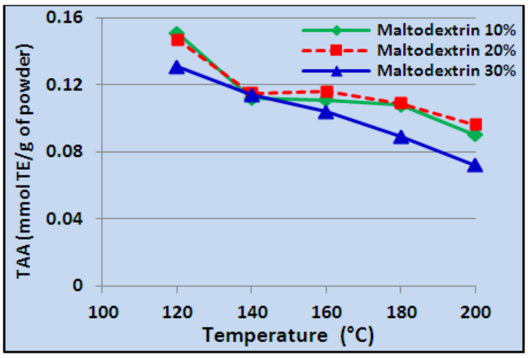

Fig. 8: Total antioxidant activity (TAA) of spray-dried powder under different dying conditions.

5. Effect of spray drying conditions on total phenolic and total antioxidant activity:

Fig. 7 shows the effect of processing conditions on total phenolic content (TPC) of spray dried powder. Drying temperature and maltodextrin concentration showed significant effect on TPC of spray dried powder. TPC was significantly (p < 0.001) reduced when inlet temperature was increased from 120°C to 160°C temperature, however above 160°C there was a reversetrend. The reason for increased TPC content in the powder above 160°C could be due to polymerization process as well as synthesis of polyphenols at 200°C which increases the totalphenolic content of the powder.TPC content of the powders was significantly reduced whenthe concentration of maltodextrin was increased from 10 to 30 %.This can be explained to be due to the concentration effect of maltodextrin.

Fig. 8 shows total antioxidant activity (TAA) of powder samples as a result of different spray drying conditions. Generally, the two factors investigated, being the maltodextrin concentration and the drying temperature, significantly affected TAA of powders ($p < 0.001$). There was no significant difference in TAA between the samples when adding maltodextrin at the concentrations of 10% and 20%. However, when the concentration increased from 20% to 30% loss of TAA was observed. Overall, increasing the drying temperature from 120 °C to 200 °C significant loss of TAA was observed, from 0.15 to 0.09mmol TE/g of powder. However, there was no statistical difference in TAA of samples spray-dried at temperaturesof 140 °C and 160 °C.

In a similar pattern to the results for the TCC of the samples, increasing maltodextrin concentration and drying temperature resulted in lower TAA of powder samples. The possible explanation is that loss of TCC at higher drying temperatures, a major antioxidant compound in spray-dried licorice powder, leads to TAA degradation. Similarly, it is also explained for a decrease of TAA as increasing maltodextrin also lowers TCC. Overall, Fig. 8 shows licorice powder that the best TAA was obtainable by using spray drying temperature of 160 °C and maltodextrin at 20% w/v.

The recommended daily intake level of carotenoids is 0.7–16.5 mg [32]. Many studies also indicate that consumption of carotenoid-rich fruits and vegetables, especially lycopene, has been linked with lower risk of prostate cancer [7,22]. Furthermore, the TCC of (1.75 mg/g) is much higher than that of other carotenoid-rich powders such as hot-air-dried pumpkin (1.14 mg/g) [33] and spray-dried watermelon powder (1 mg/g) [36]. Therefore, having established that there are high levels of carotenoids and TAA in the powder, this product is highly recommended.

From Figs. 5 and 8, similar TAA values correspond with different TCC's at different maltodextrin concentrations were observed. This demonstrates the effect of protection by the encapsulating agent. Furthermore, presence of TAA in the powders not only based on the TCC but also other antioxidative components in Licorice powders such as essential oils, glycyrrhizin and flavonoids, particularly liquiritin, isoliquiritin and their corresponding aglycones which also benefitted from the encapsulation and exert synergistic effects.

6. Scanning electron microscopy (SEM) (Particle morphology):

Fig. 9 shows the SEM micrographs of licorice powder produced with 20% maltodextrin at different inlet temperatures. It was observed that the number of particles in a given amount of the powder increased with an increase in inlet temperature. Similar findings were also reported by Tonon *et al.* [47]. Inlet temperature had no effect on the surface smoothnessof the particles. This however contradicts the observation of Allamila-Beltran *et al.* [3], Nijdam and Langrish [34] and Tonon *et al.* [47]. SEM study revealed that the average sizeof particles in the powder that was dried at higher inlet temperature was smaller than the particles in powder dried atlower inlet temperature. Similar finding was observed by Cai and Corke [6] for spray drying of amaranthusbetacyaninpigments. Probably, the particle size got fixed as large sizedglobules when there was more water in the material that wasbeing dried. At higher inlet temperature, due to rapid rate ofdrying the particles got fixed as smaller sized globules.

7. Antimicrobial activity of licorice products:

Foodborn illness resulting from consumption of food contaminated with pathogenic bacteria had been of vital concern to public health. In the same time a variety of naturally occurring plant components had been demonstrated to an antimicrobial effect as licorice.

7.1. Antimicrobial activity of licorice prodocts oninhibition zone:

The antimicrobial effect of reconstituted licorice powder from spray-dried (20% maltodextrin concentration at inlet temperature of 160 °C), and licorice (ethanolic and water) extracts at concentrations of 300, 600 and 900 μg/ml were determined against two bacterial strains gram positive (G^+),*Bacillus cereus*, and gram negative(G^-), *Escherichia coli*, one mold strain, *Aspergillusniger* and one yeast strain, *Saccharomyces cerevisiae*. The area of the inhibition zone (mm) was taken as an indicator of the antimicrobial activity.

Results in **table (2)** showed the effect of licorice products as antimicrobial agents. It was observed that the inhibitory effect of licorice increased by increasing its concentrations. It could be also noticed that the antimicrobial activity of reconstituted licorice powder reachedthe highest among its ethanolic extracts or water extracts.

Reconstituted licorice powder showed inhibition zones of 6, 8 and 12 mm against *Bacillus cereus* at concentrations of 300, 600 and 900 μg/ml respectively, while licorice ethanolic extracts showed inhibition zones of 5, 7 and 10 mm at the same concentrations respectively, but the water extracts for the same microorganism should inhibition zones of 1, 3 and 6mm at300, 600 and 900 μg/ml, respectively.

Escherichia coli was inhibited by reconstituted licorice powder at concentrations of 300, 600 and 900 μg/ml and the inhibition zones were 5, 6 and 9 mm, respectively, while ethanolic extracts of licorice at concentrations of 300, 600 and 900 μg/ml the inhibition zones were 4, 6 and 8 mm, respectively. Moreover, water extract showed no inhibition zones and hence had no antimicrobial effect against this microorganism.

Saccharomyces cerevisiae was inhibited by reconstituted licorice powder at concentrations of 300, 600 and 900 μg/ml and the inhibition zones were 3, 4 and 7 mm, respectively, while ethanolic extracts of licorice at concentrations of 300, 600 and 900μg/ml, the inhibition zones were 2, 3 and 5 mm,respectively. Water extract of licorice area was1, 2 and 3 mm at concentrations of 300, 600 and 900 μg/ml, respectively.

Aspergillusniger was also inhibited by reconstituted licorice powder at concentrations of 300, 600 and 900 μg/ml and the inhibition zones were 2, 3 and 6 mm, respectively, while ethanolic extracts of licorice, the inhibition zones were 1, 2 and 5 mm at concentrations of 300, 600 900 μg/ml, respectively. On the other hand, water extract showed no inhibition zones at all concentrations against this microorganism.

These results are in agreement with those found by Ates and Erdoğrul [5]; Statti *et al.* [43]; and Gupta *et al.* [23]. They showed that licorice ethanolic extracts had an antimicrobial activity against *Bacillus cereus* and *Escherichia coli.*

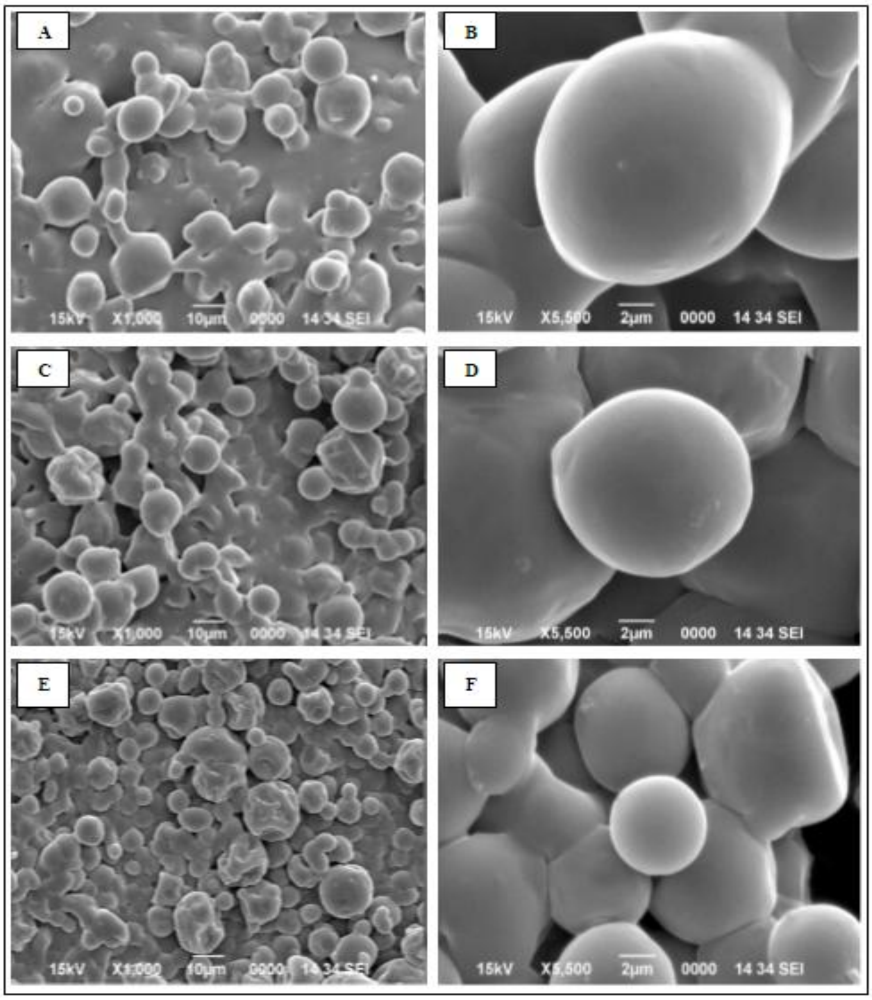

Fig. 9: Micrographs of particles at different inlet temperature and constant maltodextrin level (20%) at magnifications of (A)140°C, 1000×; (B) 140°C, 5500×; (C) 160°C, 1000×; (D) 160°C, 5500×; (E) 180°C, 1000×; (F) 180°C, 5500×.

Table 2: Effect of licorice products on the inhibition zone (mm).

Licorice products	Doses (µg/ml)	Inhibition zones (mm)			
		Bacillus cereus	*Escherichia coli*	*Saccharomyces cerevisiae*	*Aspergillusniger*
Powder (reconstituted)	300	6	5	3	2
	600	8	6	4	3
	900	12	9	7	6
Ethanolic extract	300	5	4	2	1
	600	7	6	3	2
	900	10	8	5	5
Water extract	300	1	-	1	-
	600	3	-	2	-
	900	6	-	3	-

7.2. Antimicrobial activity of licorice products on minimum inhibitory concentration (MIC):

The antimicrobial activity of reconstituted licorice powder, and licorice (ethanolic and water) extracts at concentrations 0.5, 1.0 and 1.5 % were determined against *Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli* compared with control samples as indicator of antimicrobial activity. Results in table (3) indicated that the inhibitory effect of licoriceproducts increased by increasing their concentrations. It could be noticed also that the antimicrobial activity of reconstituted licorice powder was the highest among its ethanolic or water extracts.

He *et al.* [24] found that MIC of licorice compounds were as glycyrrhizol A 1µg/ml, glycyrrhizol B 32µg/ml, 5-*o*-methylglyceryol, isoglyceryol 500µg/ml, di-isoprenyl-5,7,4-tri-hydroxy-isofjavone 2µg/ml, and gancraonin G 125µg/ml. They added that flavonoids isolated from licorice were recently reported to have antimicrobial activity against methicillin sensitive *Streptococcus mutans (MSSA), Micrococcus luteus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia and Pseodomonasaeruginosa.*

Table 3: Effect of licorice products on minimum inhibitory concentration (MIC).

Licorice products	Doses (%)	*Bacillus cereus*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Escherichia coli*
Control	–	9×10^7	3×10^7	9×10^7	8×10^7
Powder (reconstituted)	0.5	4×10^3	6×10^3	3×10^3	2×10^3
	1.0	5×10^2	3×10^3	6×10^2	7×10^2
	1.5	2×10^2	3×10^2	3×10^2	4×10^2
Ethanolic extract	0.5	9×10^4	4×10^5	7×10^4	5×10^4
	1.0	5×10^3	4×10^4	6×10^3	2×10^3
	1.5	3×10^3	3×10^3	2×10^3	1×10^3
Water extract	0.5	9×10^4	7×10^4	6×10^4	9×10^3
	1.0	6×10^3	6×10^3	6×10^3	5×10^3
	1.5	4×10^3	4×10^3	3×10^3	3×10^3

5. Conclusion:

In conclusion, the effect of spray drying conditions on the physicochemical, antioxidant properties and antimicrobial activity of licorice powders was investigated.From the observations, there was hardy powder accumulated in the collector when maltodextrin was not added in the feed. The particles produced were very sticky and mainly deposited onto the wall of drying chamber and cyclone and could not be recovered. In was observed that with the addition of maltodextrin, the condition was improved. Addition of 20% maltodextrin to the feed appeared to give better results than addition of 10% maltodextrin. These results showed that maltodextrin was a useful drying aid in spray drying of licorice aqueous solutionsand improved its yield.

Moisture content, bulk density, hygroscopicity, color characteristics, TCC, EE, TPC and TAA were significantly affected by maltodextrin concentration and by the inlet air temperatures. However, pH, $a_w$and WSI were not significantly influenced by the different spray drying conditions in this study. The licorice powder spray-dried at inlet temperature of 120 °C and maltodextrin concentration of 10% was adequately effective in preserving color, TCC and TAA. Strong positive correlations among TCC, TAA and color characteristics were also confirmed. Spray dried licorice powder made with 20% maltodextrin and processed at 160 °C inlet temperature had less hygroscopicity,a_w, bulk density, a good quality licorice powder, acceptable color, TCC and TAA.

The results obtained triggered to the effect of licorice products as antimicrobial agents. It was observed that the inhibitory effect of licorice increased by increasing its concentrations. It could be also noticed that the antimicrobial activity of reconstituted licorice powder was the highest among its ethanolic or water extracts.

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