JOURNAL OF APPLIED SCIENCES RESEARCH

ISSN:1819-544X

JOURNAL home page: http://www.aensiweb.com/JASR 2014 December; 10(13): pages 87-104.

Published Online 2014 17 July. **Research Article**

Characterization of Licorice (*Glycyrrhiza glabra*) Powders Obtained by Different Drying Processes and Evaluation of Their Antimutagenic Activity

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Received: April 23, 2014; **Revised:** May 23, 2014; **Accepted:** May June, 2014

ABSTRACT

This paper reports the effects of different drying processes (Freeze drying (FD), Spray drying (SD) and Vacuum drying (VD)) and storage on the physicochemical and antioxidant properties of licorice powders. These include moisture content, water activity (a_w),, bulk density, pH, water solubility index (WSI),, Foam volume, hygroscopicity, color characteristics, total carotenoid content (TCC), encapsulation efficiency (EE), total phenolic content (TPC), total flavonoids (TF) and total antioxidant activity (TAA). Inhibitory effects of the licorice powders on the mutagenic activity for *Salmonella typhimurium* strains TA100 and TA98 were also aimed. Moisture content, bulk density, water solubility index (WSI) hygroscopicity, color characteristics, total carotenoid content (TCC), encapsulation efficiency (EE) and total antioxidant activity (TAA) of licorice powder were significantly affected by drying technique and the storage period. However, pH, and a_w were not significantly influenced by the drying technique. All powders obtained had a high content of total phenols. Powders gained by spray drying had the highest values which corresponded to a high content of total flavonoids, Analysis of the results exhibited a correlation between selected bioactive compounds and their antioxidant capacity. The results obtained confirm that the drying technique has a strong impact on the powder quality in relation to the content of bioactive compounds. Therefore, drying parameters used should be carefully considered. In general, among drying techniques analyzed, spray drying is regarded to be the one having the smallest impact on the degradation of bioactive compounds, due to a short time and relatively soft conditions of the process. Spray drying, being the most common and cheapest (30–50 times cheaper than freeze drying) technique for the production of food materials, can be recommended for licorice powders production. Morphological study revealed that the spray dried powder had small sized particles that were densely packed. Spray dried licorice powder made with 20% maltodextrin-Arabic gum mixed and processed at 140 °C inlet temperature had less hygroscopicity, a_w, a good quality licorice powder, acceptable color, TCC and TAA. In conclusion, drying techniques have an impact on selected quality parameters, and different drying techniques cause changes in the content of bioactives analyzed. The licorice powder extracts showed antimutagenicity against BaP and MNNG in ***Salmonella typhimurium*** TA98 and TA100**.** Amounts of 3 mg per plate of licorice powder extracts were sufficient to inhibit the mutagenicity. In digested licorice powder extracts obtained by different drying processes also showed antimutagenicity. In all cases, concentration dependent inhibition was observed. All different licorice powders obtained by different drying processes in our study contained antimutagens and the extracts showed similar levels of antimutagenic activity. The antimutagenic effects of Licorice powder extracts depended on the mutagen and dose levels.

Keywords: Licorice, Freeze drying, Spray drying, Vacuum drying, Maltodextrin, Arabic gum Antioxidant properties, Anti-mutagenic activity, Encapsulation.

INTRODUCTION

Licorice (*Glycyrrhiza glabra)* roots and rhizomes are extensively used in herbal medicines for their emollient, anti-inflammatory, 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 [49].

Kitagawa [30] reported the detailed structures of 33 constituents in licorice roots and their sweetness. Glycyrrhizin is a water-soluble pentacyclic triterpenoid 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 salt form of glycyrrhizic acid (GA) which is a weak acid containing three carboxyl and five hydroxyl groups. The acid form of glycyrrhizic acid is not particularly

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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.

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 [39], or methanol, and ethanol [28]. 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 [66]. It is desirable to have it in a powder form for ease to consume its nutrients and the attractive color. Pure glycyrrhizic acid (GA) may be prepared from the licorice root by using alcohol as the solvent for extraction using an ultrasonic device followed by purification. The most common purification procedure involves acidification of the extract by adding acids such as H_2SO_4 or HCl acids [28] for the formation of the solid product of salt of GA (at pH 1–2).

The molecules of GA can also be extracted into the organic phase from aqueous solution by interacting with the polar functional groups of organic extractants through hydrogen bonding. So the nature of organic extractant and pH are the crucial factors for purification of GA. Most of the existing processes for the extraction and purification of the sweet ingredients from the licorice roots involve a number of steps and large amounts of solvents/chemicals.

Among drying techniques the most common methods applied in food industry, apart from conventional air drying, are vacuum, spray and freeze-drying [58]. Conventional air drying is one of the most widely used method for food dehydration requiring application of high temperature and long-time performance. The products obtained, when compared to the non-processed products, are generally characterised by low porosity, high apparent density and reduced quality [45]. The use of vacuum in drying systems can reduce the temperature applied for the process causing a potentially better quality of obtained products [38]. As a results of vacuum drying, the products gained have shown a better quality in relation to the retention of nutrients and aroma compounds, when compared to conventional drying [27].

Freeze drying is based on the removal of water by sublimation of the frozen product [45]. Powders obtained after freeze drying are generally characterised by low bulk density, high porosity as well as good aroma and taste retention [31]. However, a disadvantage of this process is the long time of drying and, consequently, high energy consumption. An industry scale comparison showed that the freeze drying process is 4–5 times more expensive when juxtaposed with spray drying, and approximately nine times more expensive than single-stage evaporation process [23]. It has to be noted, however, that high-tech technologies can considerably reduce the costs of the processes.

Spray drying is a one-step processing operation with minimum handling, in which liquid products are turned directly into fine powders [10]. This process is broadly applied in fruits, vegetables and natural drinks industry [3] due to many advantages of the powders gained, such as good quality and low water activity and are suitable for transportation and storage. Furthermore, it is a highly appropriate process for heat sensitive components such as carotenoids [55]. On the other hand, there can also be some negative aspects of this type of drying namely stickiness and hygroscopicity of products influenced by the presence of low molecular weight sugars and acids [22,32].

There are different methods for encapsulation in the food industry. Freeze drying which has a long dehydration period has been used as a simple technique in encapsulating water-soluble essences and natural aromas or drugs. During this procedure, core materials and matrix solutions are homogenized and then co-lyophilized to make dried materials [18]. Several additives such as maltodextrin, Arabic gum 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 [14]; carrot carotenes [61]; guava juice [11] and pineapple juice [1]. Furthermore, color of foods is one of the most important sensory attributes which is affected by many factors during drying process such as the temperature and additives [1]. However, little information is published on preserving of Licorice drinks and no study on different drying techniques using maltodextrin and/or Arabic gum as the carrier/encapsulating agent has been reported for producing Licorice powder.

In general, the quality of dehydrated products is mostly characterized by physical properties such as appearance, texture, color and porosity, but also by the retention of nutrients present in non-processed fruits. It is well known that drying processes provoke changes in the quality and quantity of bioactive compounds and their antioxidant capacity [34,45]. It is worth to mention that information about the exact alterations in chemical composition of licorice subjected to drying processes is still limited. Complexity of this issue applies to a wide range of drying techniques including their different variations and options. However, until now data on licorice

powders obtained by different drying techniques have been insufficient. Thus, the aim of the present study was to assess different drying methods and storage on the basis of the content of bioactive compounds in licorice powders.

This paper reports the effects of different drying techniques and storage on the physicochemical and antioxidant properties of licorice powder. These include moisture content, water activity, bulk density, pH, water solubility, hygroscopicity, color characteristics, carotenoid content, encapsulation efficiency, total phenolic content, total flavonoids and antioxidant activity. Inhibitory effects of the licorice powders on the mutagenic activity for *Salmonella typhimurium* strains TA100 and TA98 were also aimed. Furthermore, we also investigated the antimutagenic activity of Licorice extracts after digestion in simulated gastric and intestinal juice under various conditions with mutagen. The aim of the present study is a need to know the absorption of flavonoids and phenolic acids whether they are loss or not in the simulated gastric and intestinal juices. If they will absorb and metabolize, they have been shown to inhibit about mutagen.

Materials and Methods

Materials:

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%), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium), chlorogenic acid and quercetin were purchased from Sigma–Aldrich (Louis, USA). Maltodextrin DE 12 (MD) and Arabic gum (AG) were purchased from Glucidex®, (Roquette, France).

Pepsin (from hog stomach), pancreatin (from porcine pancreas), Benzo[*a*]pyrene (BaP) and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) were purchased from Wako Chemical Inc. Aroclor 1254-induced hepatic S9 was made for the activation system in the case of BaP and MNNG. Agar and Nutrient Broth No.2 were purchased from the Difco Laboratories (Detroit, USA) and Oxoid (Hampshire, UK), respectively. All general chemicals used in this study were of analytical grade.

2. Microorganisms:

The *Salmonella typhimurium* strains TA98 and TA100 and liver S9 were obtained from Department of Cancer Biology, National Cancer Institute, Cairo, University.

3. Licorice (or Liquorice) Root Preparation:

3.1. Licorice Root (Glycyrrhiza glabra) 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, then sonicated for 3 min. 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 until use.

3.2. Drying Processes:

Portioned licorice extracts were subjected to drying processes: different ratios of maltodextrin (12 DE) and Arabic gum mixed (2:1) were added into the licorice water extracts, which were blended and finally filtered before drying process. The three ratios of maltodextrin-Arabic gum mixed (2:1) 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. Feed material for all the runs had to come from one master batch to be consistent, so there was just enough for duplicated runs.

3.2.1. Spray Drying (SD):

Preliminary spray drying trials showed that when the concentration of maltodextrin (12 DE)-Arabic gum mixed (2:1) was lower than 10%, there was pumping problem and most materials stuck on chamber wall; when the concentration of maltodextrin-Arabic gum mixed (2:1) was higher than 30%, the resulting powder lost its attractive color.

The feed mixtures comprising added maltodextrin-Arabic gum mixed 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 140 °C/94 °C. The drying air flow rate, compressor air pressure and feed rate were constant. 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].

3.2.2. Freeze Drying (FD):

Freeze drying was carried out using a laboratory freeze dryer (Freeze Mobile 24, Virtis Company, Inc., Gardiner, NY). The licorice aqueous solutions was poured into a stainless samples pans (200 mm

diameter – 10 mm height). The samples were placed at –40 °C for 24 h before transferring to the freeze dryer. The vacuum pressure of the dryer was set at 20 Pa, the plate temperature was 20 °C, and the condenser was at –60 °C. The residence time needed to dry the licorice aqueous solutions to below 5 g water/100 g dry solids was determined when the vacuum pressure had dropped to 30 m Torr (4 Pa). Powders were pulverized by mill using the sieve of 1.0 mm diameter (Retsch SM-100, Hann, Germany) [35].

3.2.3. Vacuum Oven Drying (VD):

The vacuum oven drying was employed according to the procedure described by Wang *et al.* (2010) with some modification. As mentioned earlier, the maltodextrin-Arabic gum mixed with licorice extracts (20%, w/v) was prepared and then homogenized using the same homogenization process (at 30 and 25 MPa). Then, the homogenized maltodextrin-Arabic gum solution was dried by using a vacuum-dryer at 60 °C for 24 h. The vacuum and temperature were maintained at 5 psi and 60 °C, respectively. The dried sample was then milled and passed through a 1.0 mm sieve. Finally, the milled powders were packed in high barrier aluminum–plastic laminated bags and stored at 4±1 °C until the analysis [40].

4. Storage stability evaluation:

Samples of each of the dry powders obtained from different drying techniques were stored at room temperature in metalized bags [55] to determine the effect of storage on characterization of licorice powders. Degradation of licorice powders was monitored for 15 weeks and the licorice powders was analyzed at 0, 3, 6, 9, 12 and 15 weeks of storage according to Lee and Durst [33].

Analytical Methods:

All analytical measurements were carried out in triplicate.

1. Water Content (WC):

Water content in licorice powder was performed with HG53 Halogen Moisture analyzer (Mettler, Toledo, Spain). Furthermore, water content in powders was carried out by Karl-Fischer method using 803 KF Titration stand (Metrohm, Herisau, Switzerland).

2. Water Activity (a_w):

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

3. pH Determination:

The pH value of licorice powders was determined for pH by blending 5 g powder with 25 ml deionized water at 20°C, using the pH meter calibrated with standard buffers pH 7 and 4.

4. Water Solubility Index (WSI):

The WSI of the licorice powders was determined using the method described by Mukhopadhyay and Panja [39]. Dried licorice powders by different drying processes (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.

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 [20].

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].

7. Foam Volume:

Powder samples were used in rehydrated form. To exclude the confounding effect of differences in the soluble solid contents of the samples on foam formation and to ensure a reliable comparison, all powder samples were reconstituted to 10°Bx. The reconstituted extracts were treated for three minutes with Ultra Turrax T25 (Alberta, USA) at 15000 rpm in a 250 ml graduated cylinder, and the foam volume was directly measured [4].

8. Color Characteristics:

Licorice powder samples were analyzed for Hunter L^*, a^* and b^* color with a Hunter Lab Scan XE Colorimeter (Hunter Laboratory Inc. Restonva). The instrument was standardized each time with a black and a white (L = 91.10, a = –1.12, b = 1.26) tile. The color values were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness) and ΔE (total color difference). In each treatment, the reflectance measurement was obtained from the average of six readings. Samples were evaluated in triplicates.

Chroma, indicating color intensity, was calculated by the formula $(a^{*2} + b^{*2})^{1/2}$. 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:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where L_0^*, a_0^* and 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 [15].

9. Determination of Total Carotenoids Content (TCC):

A method described by Tran *et al.* [57] was employed, with some modifications, to extract the carotenoids 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 NaCl solution were added to the funnel to facilitate phase separation. The upper layer was collected to measure total carotenoids content. The process was conducted under dim light and analyzed within one day.

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).

10. Encapsulation Efficiency (EE):

A method described by Shu *et al.* [51] was employed, with some modifications, to calculate encapsulation efficiency. The EE (%) was determined as the ratio between the initial content of total carotenoids before drying processes and the content of the final powder product (mg/ g powder).

11. Total Phenolic Compounds (TPC):

Estimation of total phenolic content was performed by Folin–Ciocalteu method described by El-Hamzy *et al.* [16] with some modifications. Briefly, 250 mg of sample was 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) / g of licorice powder (DM).

12. Total Flavonoids (TF):

The TF content was determined according to the method of El-Hamzy *et al.* [16]. Briefly, aliquots of 25 µL of extracts were mixed with 75 µL of 95% ethanol (v/v). Subsequently, 5 µL of 10% $AlCl_3$ *6H2O as well as 5 µL of 1 mol/L potassium acetate were added by the injector followed by the addition of 140 µL of deionised water. The absorbance of supernatants was recorded at 415 nm against deionised water after 30 min. The results obtained were expressed as mg quercetin (Q) per g of licorice powder DM.

13. Determination of Total Antioxidant Activity by The ABTS Assay:

The Trolox Equivalent Antioxidant Capacity (TEAC) assay was performed as described by Horszwald and Andlauer [24]. Aliquots of 10 µL sample were placed into microplate wells followed by the addition of 290 µL of $ABTS^{\bullet+}$ [2,2'-azino-bis (3-ethylbenzthiazonline-6-sulfonic acid)] solution (after incubation with $K_2S_2O_8$) by the microplate reader's injector. The reaction was carried out at 30 °C in dark during 6 min. After this time, the values of absorbance were recorded at 734 nm. The results obtained were expressed as mmol Trolox Equivalents (TE) per g of licorice powder (DM).

14. Scanning electron micrograph:

Particle morphology was evaluated by scanning electron microscope *(SEM)*. Powders were attached to a double sided adhesive tape on SEM stubs, coated with gold 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 300×, 1000× and 2500×.

Antimutagenic activity:

1. Preparation of the gastric and intestinal juice:

The simulated gastric and intestinal juice was prepared as follows (USP 23).

The gastric fluid consisted of 2.0 g of NaCl and 3.2 g of Pepsin, dissolved in 7.0 ml of HCl, and water was added to make 1000 ml. The pH of the gastric fluid was pH 1.2. The intestinal fluid consisted of 6.8 g of KH_2PO_4, dissolved in 250 ml of water, 190 ml of 0.2N NaOH, 400 ml of water and 10.0 g of pancreatin. The pH of the intestinal fluid was pH 7.5±0.1.

2. Preparation of digested sample solution:

The licorice powder extracts were each submitted to gastric or intestinal juice and a mutagen such as benzo[*a*]pyrene (BaP) or *N*-methyl-*N*'-nitro-*N*-nitroso-guanidine (MNNG). A 0.1 ml aliquot of each solution of extract and mutagen was subjected

to an equal volume of gastric juice for 30 min. Another 0.1 ml aliquot of each solution of extract and mutagen submitted to an equal volume of intestinal juice for 2 h. This process was performed in triplicate. Digested samples were stored at –20 °C until analysis in the mutagenic activity.

3. Antimutagenic activity of licorice powder extracts:

The antimutagenic activity of the licorice powder extracts used in the experiments with the in vitro model was tested by Ames test [36]. The antimutagenicities were used in the concentration of 3 mg per plate. As a mutagenic agent for TA98 and TA100 was 0.2 µg for BaP and 0.1 µg for MNNG. For each sample three agar plates were used and the number of revertants per plate was counted. Results of the Ames test were expressed as mean number of revertants per triplicate plates, corrected for spontaneous revertants.

4. Antimutagenic activity of digested licorice powder extracts:

The antimutagenic activity of the digested licorice extracts used in the experiments with the in vitro model was tested by Ames test [36]. This procedure is same that used to measure antimutagenic activity of the licorice powder extracts. All experiments were performed in triplicate. Data presented in the tables are means from three independent series.

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).

Results and Discussion

1. Effect of Maltodextrin-Arabic Gum Mixed:

From the observations, there was hardy powder accumulated in the collector when maltodextrin-Arabic gum mixed was not added to the feed. The particles produced were very sticky and mainly deposited onto the wall of drying chamber could not be recovered.

Fig. 1: (A and B) Spray drying with (A) 10% and (B) 20% maltodextrin-Arabic gum mixed (2:1).

Therefore, maltodextrin-Arabic gum mixed (2:1) 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 12. Other researchers have reported that low DE maltodextrin had better nutrient binding properties [47]. In was observed that with the addition of maltodextrin-Arabic gum mixed (2:1), the condition was improved. Addition of 20% maltodextrin-Arabic gum mixed to the feed appeared to give better results than addition of 10% maltodextrin-Arabic gum mixed (Fig. 1: A and B). These results showed that maltodextrin-Arabic gum mixed was a useful drying

aid in spray drying of licorice aqueous solutions as it improved the yield of product.

2. Effects of different drying processes on the physicochemical properties of Licorice powder:

The moisture content of the powders prepared was noted to be highly dependent on the parameters selected and fixed for each drying method. Due to the varying water content of the dried products and for a better comparability, all the results obtained were calculated on a dry matter (DM) basis.

The effects of different drying processes using 20% maltodextrin-Arabic gum mixed (2:1) as wall material on the physicochemical properties of licorice powders are shown in Table 1. Results showed that spray drying technique (VD) of licorice extract was gained lower percent of moisture content (2.09%) while, freeze drying technique (FD) was gained highly percent of moisture content (4.92%). Similarly, Rodríguez-Hernández *et al.* [47]; and Ersus and Yurdagel [17] reported that the moisture content of tomato powder, orange juice powder, cactus pear juice powder and black carrot powder decreased as drying temperature increased and to be highly dependent on the parameters selected and fixed for each drying technique.

For spray drying technique in general, resulted in greater loss of water of resultant powder, due to the higher rate of heat transfer into particles, causing faster water removal. A similar result was also reported by Grabowski *et al.* [22] 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.

Table 1: Drying processes parameters of licorice powders and characteristics of powders obtained by different drying techniques.

Drying technique	WC (%)	a_w	pH	WSI (%)	Bulk density (g/ml)	Foam Volume (ml)	Hygroscopicity [g (AM)/100g]
Freeze Drying	4.92 ± 0.51^{c}	0.53 ± 0.05	5.35 ± 0.15	86.25 ± 3.01^{a}	0.79 ± 0.15^{c}	83.50 ± 2.51^{a}	65.25 ± 1.09^{c}
Spray Drying	2.09 ± 0.44^{a}	0.41 ± 0.09	5.19 ± 0.11	96.45 ± 1.32^{c}	0.60 ± 0.19^{a}	101.25 ± 4.19^{c}	44.22 ± 0.13^{a}
Vacuum Drying	2.21 ± 0.39^{b}	0.43 ± 0.07	5.21 ± 0.16	91.51 ± 2.22^{b}	0.68 ± 0.11^{b}	97.33 ± 1.13^{b}	48.34 ± 0.18^{b}

Values are mean ± SD (two replicates) after statistical analyses.
The values in the same column followed by different superscripts (a–c) were significantly different ($p < 0.05$).
AM: adsorbed moisture.

The values of pH and a_w of the licorice powders in this study were not significantly affected by different drying processes ($p > 0.05$). For pH value, this finding is in agreement with the results of Gonzalez-Palomares *et al.* [19] who found that pH of the Roselle extract powders did not change with different drying processes. 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 [54]. The average a_w of powders in this study ranged from 0.41 to 0.53 by using SD and FD drying techniques, respectively (Table 1), and could be considered to be quite microbiologically stable. The results for a_w of the powders were consistent with the findings of the study carried out by Quek *et al.* [44]. They stated that the water activity of spray-dried watermelon powders reduced from 0.44 to 0.40 by using inlet temperatures between 145 °C and 175 °C.

The WSI of samples in the study was influenced by different drying processes ($p < 0.05$). A similar observation was also reported by Sousa *et al.* [52] 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 freeze dried tomato powders, which ranged from 17.65% to 26.73%. However, the licorice WSI values were much higher compared 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 [2,20]. 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 [13]. Therefore, further investigation may need to be carried out to identify methods for improving the water solubility of licorice powders further when 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 [21,8]. 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 [29,8].

The foam producing capability of the reconstituted licorice powder is important as a special product characteristic due to the presence of saponins in the composition, which are glycosides having foam producing behavior. The foam volume of licorice extract having 10 °Brix soluble solid content was measured to be 100 ml whereas the foam volume of reconstituted powders obtained by different drying processes ranged from 83.5 ml to 101.25 ml. According to the results, a higher increase

in the foam volume of licorice extract (101.25 ml) in dried powders by using SD technique followed by VD technique (97.33 ml).

Considering the feed's soluble solid content, it can be stated that powders having relatively higher foam-producing capacities were obtained at 22.5°Brix, and an increase or decrease of the feed's soluble content decreased the foam volume. This behavior indicates that maltodextrin degrades to oligosaccharides, disaccharides and glucose during reconstitution of the powder prior to the foam-producing capacity measurements. In industry, sugar syrups such as glucose syrup are used as supporters of foam production. The comparison between the foam producing capacities of licorice extract with 10°Brix and reconstituted powder of the same extract (foam volume of 100.33 ml) indicates that drying operation does not have any effect and considering the advantages of powder product compared to liquid extract shows the suitability of the spray drying operation.

The difference of drying technique had significant ($p < 0.05$) effect on the hygroscopicity of the licorice powder. Hygroscopicity was the lowest when SD technique was used for encapsulation. Drying temperature also influenced the hygroscopicity of the powder significantly. The highest hygroscopicity value of 65.25 g/100g for licorice powder was obtained when using FD technique. When temperature of processing was increased the hygroscopicity of licorice powder was decreased. Rodriguez-Hernández *et al.* [47]; and Cai and Corke [6] also observed a reduction in hygroscopicity when using SD technique of cactus pear juice. Maltodextrin is a material having the property of low hygroscopicity and its utility as a carrier material for spray drying [55] has been established.

The present findings are in agreement with Moreira *et al.* [37] but contradicts the findings of Goula *et al.* [20] and Tonon *et al.* [55] 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 different drying processes and storage at room temperature on the color characteristics of Licorice powder:

The differences between colors of the powders obtained after different drying processes are presented in Figs. 2 and 3. Lower L^* values gained for powders obtained after oven vacuum drying showed a darker color when compared to the powders gained after freeze and spray drying. Positive a^* values for licorice powders associated with orange color were inconsistent with empirical observations. A further characterization exhibited that all samples presented positive b^* values (indicating hue on yellow axis), showing significant differences among drying techniques oven vacuum drying, spray and freeze drying. Parameter ΔE (Fig. 4) reflects a total color difference and confirms that powders gained after oven vacuum drying were similar, in contrast to the ones obtained after freeze or spray drying.

Figs. 2 and 3 also show the effects of different drying processes and storage at room temperature on the color characteristics of licorice-dried powders. In general, the color characteristics of licorice-dried powders were significantly impacted by different drying processes and storage period. For the lightness (L^*), the color of products was significantly affected by different drying processes ($p < 0.01$). An increase in the lightness of products was significantly between VD and SD techniques. However, there was no significant difference in lightness of dried samples between SD and FD techniques. 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 dried sample by using VD technique, both indicating more yellowness. Moreover, the different drying processes also impacted on the chroma value of samples ($p < 0.001$). Decreasing the chroma from 31.23 ± 2.56 to 25.55 ± 3.19 was observed when using VD and SD techniques, respectively.

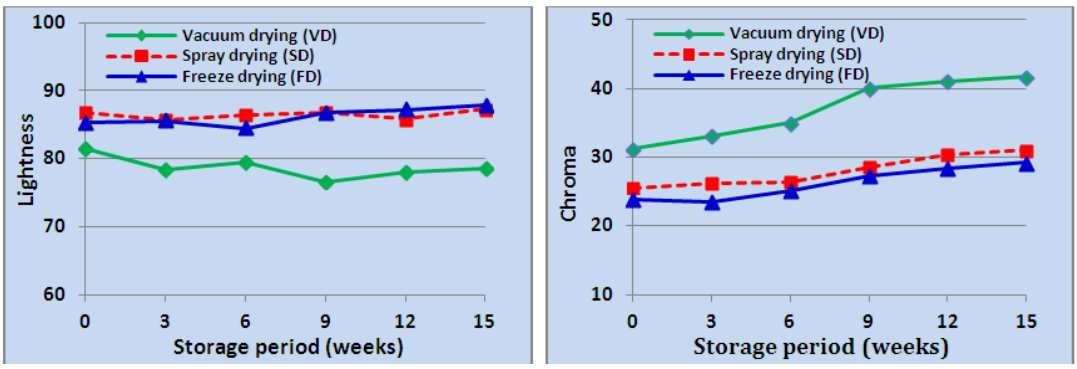

Fig. 2: The lightness and chroma of licorice powders as a result of different drying processes and during storage at room temperature.

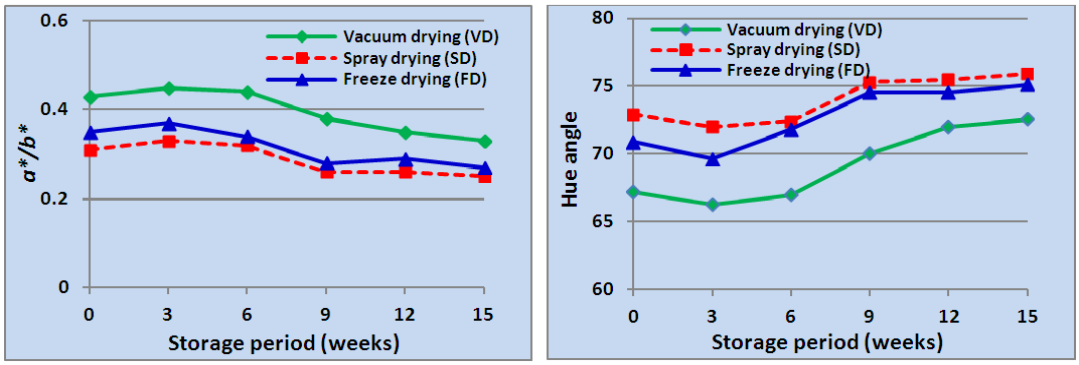

Fig. 3: The ratio a*/b* and hue angle of licorice powders as a result of different drying processes and during storage at room temperature.

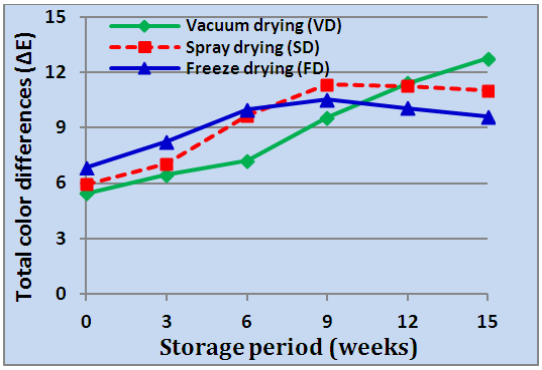

Fig. 4: Total color differences of reconstituted Licorice Powders after different drying processes and during storage at room temperature.

Storage period was another factor affecting the color characteristics of products, namely chroma, hue angle and *a*/b** value, but not lightness. A significant effect of Storage period on the *a*/b** value and hue angle was statistically observed ($p < 0.01$). Loss of yellowness of samples dried by VD technique, resulting in low *a*/b** value and high hue angle, increased when increasing storage period from 3 to 9 weeks; however, no statistical difference in the value of *a*/b** and hue angle between 9, 12 and 15 weeks of storage. In contrast, the lightness of products was not significantly influenced by storage period in different drying processes ($p > 0.05$).

The total color difference *(ΔE)* of reconstituted licorice powders after different drying processes compared to feed mixtures before the drying processes is shown in Fig. 4. The Δ*E* of reconstituted licorice powders was not impacted by different drying processes ($p > 0.05$); however, a significant effect of storage on Δ*E* was statistically observed ($p < 0.001$). Increasing storage time weekly significantly resulted in an increase in Δ*E*. Moreover, there was no significant interaction between different drying processes and storage period.

Generally, an increase in the lightness value of the powders was observed when using spray drying technique. Similar results were also found in spray-dried sweet potato powders [22] and in pineapple juice powders [1]. On the other hand, the lightness of licorice powders in this study was not significantly influenced by storage time for 15 weeks. However, Sousa *et al.* [52] 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 the high content of sugar causing browning of powders [44].

The chroma value of licorice powders was significantly affected by both the different drying processes and storage time. High chroma value of powders was observed when using VD technique and storage time for 15 weeks. 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.* [44].

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 storage time; however, not by the different drying processes. 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 increasing storage time. Additionally, it can be clearly seen that total color differences (Δ*E)* is a function of value *L**

a b**, therefore, the increase in lightness with increased storage time was also a contribution to increasing Δ*E*. Contradictorily, Rodríguez-Hernández *et al.* [47]; and Grabowski *et al.* [22] indicated that the influence of drying processes was found to be significant for the variation of Δ*E* 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 different drying processes and storage at room temperature on the total carotenoids (TCC) and encapsulation efficiency (EE):

The total carotenoids content (TCC) and encapsulation efficiency (EE) of the licorice powders as a result of different drying processes are presented in Figs. 5 and 6, respectively. The TCC in powder samples reduced from 1.87 mg/g to 1.21 mg/g of powder when using FD and VD technique, respectively. Further, the storage period also affected TCC; significant loss of TCC in samples was observed as storage period increased from 3 to 15 weeks ($p < 0.001$). However, there was no statistical difference in TCC of samples storage between 6 to 9 weeks. Overall the best TCC retained was found with using FD and SD techniques.

According to Goula and Adamopoulos [20], an increase in storage period resulted in a greater loss of lycopene content in tomato powders. Similarly, Quek *et al*. [44] observed that a decrease in the lycopene and β-carotene content of spray dried watermelon powder occurred as a result of increasing the storage period. The main reason for these findings was due to thermal degradation and oxidation. In addition to the storage period, the loss of carotenoids in the licorice powder samples was also dependent on several factors, such as drying temperatures, droplet moisture content, oxygen and exposure to light. These factors are governed by processing conditions such as initial feed solid concentration, drying technique and temperature. Moreover, 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 [20,21]. 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 [44].

The encapsulation efficiency (EE) of the study samples was also significantly influenced by drying technique and storage period ($p < 0.001$). However, no difference in EE between FD and SD techniques of licorice extracts was observed. Moreover, in general, EE of the samples reduced from 86.95 % to 64.11 % and 86.22 to 68.99 % for FD and SD techniques as the storage time increased from 3 to 15 weeks, respectively.

In terms of the effect of storage period, a similar pattern to the TCC loss as a result of increasing the storage period was also observed in relation to EE. This indicated that increasing storage time 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.* [51], the balance between the rate of water evaporation and film-formation may break due to increasing storage period; therefore, wall system of microcapsules is broken down. This phenomenon will cause a low EE. Similar findings were also reported by authors [51,53]. This is well known that carotenoid content in powder is effectively protected at a high initial feed solid. Similar observations were found in other study [47].

5. Effect of different drying processes and storage at room temperature on total phenolic compounds (TPC), total flavonoids (TF) and total antioxidant activity (TAA):

5.1. Total Phenolic Compounds (TPC):

Fig. 7 shows the effect of different drying process on total phenolic compounds (TPC) of licorice dried powder. The content of total phenolic compounds in licorice powders was from 276.53 mg CAE/100 g to 329.28 mg CAE/100 g DM. The values obtained were considerably higher when compared to TPC content in other dried licorice products [26], suggesting a strong influence of the method of powders preparation. Similarly, the content of total polyphenolic compounds in licorice powders obtained after processes analyzed was significantly higher when compared to other data regarding licorice before dying processes [43]. Application of drying processes caused changes between TPC in the powders obtained. A less strong influence of the drying process was observed in the case of spray drying contrary to the previous conclusion that utilization of this technique caused the smallest alterations in the content of phenols present in processed licorice extracts [25]. This finding indicates that spray-drying technique preserves higher levels of total phenolic compounds when compared to other techniques. The strongest influence on total phenols compounds was noted for freeze drying, where the value was nearly 20% lower when compared to spray drying. However, no statistical differences have been observed. Application of drying in low temperatures led to the degradation of polyphenols, which is understandable considering the drying time of 140 h.

Fig. 7 also shows the effect of storage period on total phenolic compounds (TPC) of licorice dried powder. Storage period showed significant effect on TPC of licorice dried powder. TPC was significantly ($p < 0.001$) reduced when storage period was

increased from 3 to 9 weeks, however above 9 weeks there was a reverse trend. The reason for increased TPC content in the powder above 9 weeks of storage could be due to polymerization process as well as synthesis of polyphenols which increases the total phenolic compounds of the powder.

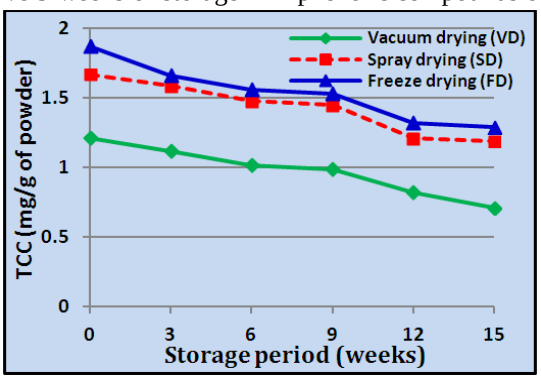

Fig. 5: Total carotenoids content (TCC) of Licorice powders as a result of different drying processes and during storage at room temperature.

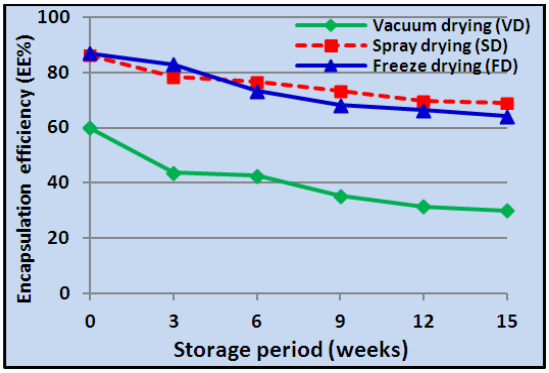

Fig. 6: Encapsulation efficiency (EE %) of Licorice powders as a result of different drying processes and during storage at room temperature.

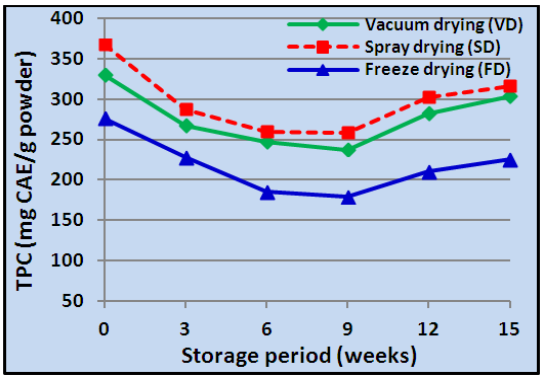

Fig. 7: Total phenolic content (TPC) of Licorice powders as a result of different dying processes and during storage at room temperature.

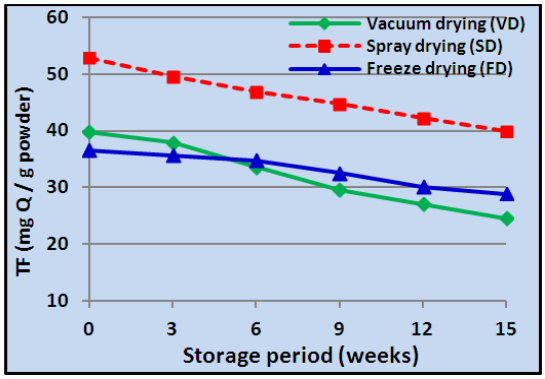

Fig. 8: Total flavonoids (TF) of Licorice powders as a result of different dying processes and during storage at room temperature.

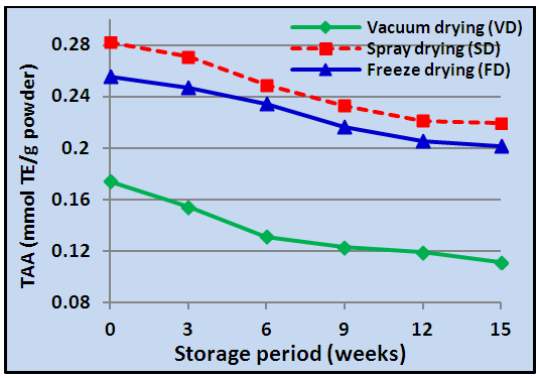

Fig. 9: Total antioxidant activity (TAA) of Licorice powders as a result of different dying processes and during storage at room temperature.

5.2. Total Flavonoids:

The content of total flavonoids (TF) was on the similar level between powders analyzed with the average value of 39.68 mg quercetin (Q) / g licorice powder (DM) except spray drying (Fig. 8). This process provokes the weakest alteration in the content of total flavonoids. In comparison to the average value obtained for the powders analyzed, the TF content in powder after spray drying was higher of approx. 40%. In general, a tendency related to the composition of the powders was similar to those obtained for total phenols. Usually, drying processes lead to the degradation of flavonoids that are strongly dependent on the drying method [25]. Assumptions made in this study stand in opposition to the previous statements that freeze drying is a less aggressive technique for flavonoids preservation in comparison to hot air and oven vacuum drying [67]. A strong influence of the relatively long drying time on TF was noted, confirming that the loss of such macromolecules might be caused by the combination of the duration and temperature [48].

5.3. Total antioxidant activity by ABTS:

The results presented in Fig. 9 indicated differences in the antioxidant activity between powders analyzed, suggesting the influence of drying parameters on ABTS values. The lowest ability to scavenge $ABTS^{+}$ radical cations was noted for powders obtained after vacuum drying technique (VD) followed by freeze drying (FD). The spray drying (SD) process led to the weakest changes in the concentration of the compounds that are able to scavenge ABTS radicals. The results obtained were about 40% higher when compared to the average value for VD technique.

Fig. 9 also shows the effect of storage period on total antioxidant activity (TAA) of powder samples as a result of different drying techniques. Generally, the two factors investigated, being the drying techniques and the storage, significantly affected TAA of powders ($p < 0.001$). There was no significant difference in TAA between the samples when using SD and FD technique. However, when the concentration increased from 20% to 30% loss of TAA was observed. Overall, increasing the storage period from 3 to 15 weeks significant loss of TAA was observed, from 0.28 to 0.11 mmol TE / g of powder.

From Figs. 5 and 9, similar TAA values correspond with different TCC's in different drying techniques 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. 10 shows the SEM micrographs of licorice powders produced by different drying techniques with 20% maltodextrin-Arabic gum mixed (2:1). It was observed that the number of particles in a given amount of the powder increased by using spray drying technique. Similar findings were also reported by Tonon *et al.* [55]. Inlet temperature had no effect on the surface smoothness of the particles when using spray drying and vacuum drying techniques. This however contradicts the observation of Allamila-Beltran *et al.* [3], Nijdam and Langrish [42] and Tonon *et al.* [55]. SEM study revealed that the average size of particles in the powder that was dried by using spray drying technique [Fig. 10: B (1, 2 and 3)] was smaller than the particles in powder dried by using vacuum drying technique [Fig. 10: C (1, 2 and 3)]. Similar finding was observed by Cai and Corke [6] for spray drying of amaranthus beta-cyanin pigments. Probably, the particle size got fixed as large sized globules when there was more water in the material that was being dried. At higher inlet temperature (spray drying technique, 140 °C), due to rapid rate of drying the particles got fixed as smaller sized globules.

Microcapsules obtained from vacuum drying powders using maltodextrin-Arabic gum mixed (2:1) in level 20% were found to be nearly spherical but had many dents on the surface, whereas the microcapsules obtained from freeze drying technique [Fig. 10: A (1, 2 and 3)] using the same wall material were broken and not complete. A comparative study on gum Arabic and modified starch for encapsulation of flavors showed a similar pattern of microcapsules [5,59]. The SEM also indicated the suitability of Spray drying technique using maltodextrin-Arabic gum mixed (2:1) in level 20% as a wall material for encapsulation of licorice extracts as compared with freeze drying or vacuum drying techniques.

7. Antimutagenic activity of Licorice powder and digested Licorice powder extracts obtained by different drying processes:

The licorice powder extracts showed antimutagenicity against BaP and MNNG in *Salmonella typhimurium* TA98 and TA100 (Table 2). Amounts of 3 mg per plate of licorice powder extracts were sufficient to inhibit the mutagenicity induced by any of the BaP (Benzo[*a*]pyrene) and MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) used concentrations. These results suggest that ethyl acetate extracts have antimutagenic effects on both BaP and MNNG. Licorice extracts has already demonstrated its effective antimutagenic activity [63]. In Tables 3 and 4, digested licorice powder extracts obtained by different drying processes also showed antimutagenicity. In all cases, concentration dependent inhibition was observed. There were no significant differences in antimutagenicity on the digested licorice powder extracts. All different licorice powders obtained by different drying processes in our study contained antimutagens and the extracts showed similar levels of antimutagenic activity. The antimutagenic effects of Licorice powder extracts depended on the mutagen and dose levels.

Licorice polyphenols may be responsible for the antioxidant activity of licorice. Glabridin, one of the major flavonoids in licorice extracts (11.6%, wt/wt), and its isoflavan derivatives were shown to be potent antioxidants against β-carotene consumption and 2,2 β-azobis (2-amidinopropane) dihydrochloride-induced low-density lipoprotein (LDL) oxidation.

Licorice powder extracts maintained their antimutagenic potential in vitro simulated digestion model. Thus, antimutagenic compounds from licorice powder extracts were not inactivated by gastric acid and intestinal juices and were readily available for absorption.

Table 2: Antimutagenic activity of licorice powder extracts obtained by different drying processes against BaP (0.02 µg, 0.2 µg/plate) and MNNG (0.1 µg, 0.01 µg/plate) on *Salmonella typhimurim* TA 98 and TA 100.

Licorice (3mg/plate)	BaP				MNNG			
	Revertants/plate[a]		Percent inhibition (%)		Revertants/plate		Percent inhibition (%)	
	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100
Control	165.6±2[b]	298.2±5	0	0	151.1±7	290.2±4	0	0
FD	59.9±6	144.5±2	63.8±2.1	51.5±1.2	64.2±10	120.1±9	57.5±2.5	58.6±3.6
SD	46.1±2	117.2±9	72.2±0.5	60.7±1.1	56.9±4	98.2±11	62.9±1.7	66.2±1.1
VD	55.6±7	131.1±7	66.4±0.8	56.0±2.5	60.4±8	111.0±5	60.0±2.3	61.7±0.4

[a]: Triplicate plates were tested per dose per experiment.
[b]: Datas are presented as the mean ± S.D of triplicate determinations.
BaP : Benzo[*a*]pyrene. MNNG : *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine.
FD: Freeze drying. SD: Spray drying. VD: Vacuum drying.

Table 3: Antimutagenic activity of licorice powder extracts obtained by different drying processes against BaP (0.02 µg, 0.2 µg/plate) on *Salmonella typhimurim* TA 98 and TA 100 under simulated gastric juice or intestinal juice.

Licorice (mg/plate)	Gastric juice				Intestinal juice			
	Revertants/plate[a]		Percent inhibition (%)		Revertants/plate		Percent inhibition (%)	
	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100
Freeze Drying								
Control	191.5±6	177.0±3	0	0	229.0±6	286.6±3	0	0
1	95.3±10	123.2±7	50.2±1.3	30.4±0.4	116.1±10	119.4±14	49.3±2.0	58.3±0.9
2	70.1±9	88.9±10	63.4±0.7	49.7±1.1	68.2±12	113.5±9	70.2±0.7	60.4±0.8
3	30.5±5	66.1±8	84.1±0.4	62.7±1.7	57.7±15	94.4±4	74.8±0.8	67.1±1.6
Spray Drying								
Control	239.6±2[b]	318.4±4	0	0	238.5±9	496.2±7	0	0
1	94.9±6	241.1±9	60.4±7	24.3±1.0	176.0±4	190.3±10	26.2±0.9	61.7±1.6
2	54.3±10	159.0±11	77.3±1.2	50.1±0.7	99.3±12	171.2±6	58.4±1.7	65.5±1.1
3	24.0±12	107.9±13	90.0±1.5	66.1±0.7	38.1±9	115.5±6	84.1±2.0	76.7±0.6
Vacuum Drying								
Control	155.5±11[b]	276.2±6	0	0	140.3±10	282.4±6	0	0
1	64.9±4	147.8±2	56.5±2.9	46.5±2.2	63.0±8	125.7±4	55.1±2.5	55.5±3.6
2	58.3±9	137.5±12	62.5±0.8	50.2±3.1	59.2±6	117.3±7	57.8±2.3	58.6±0.4
3	51.3±10	120.2±10	67.5±0.5	56.5±1.4	55.2±3	105.5±9	60.6±1.7	62.7±1.1

[a] Triplicate plates were tested per dose per experiment.
[b] Datas are presented as the mean ± S.D of triplicate determinations.
BaP: Benzo[*a*]pyrene.

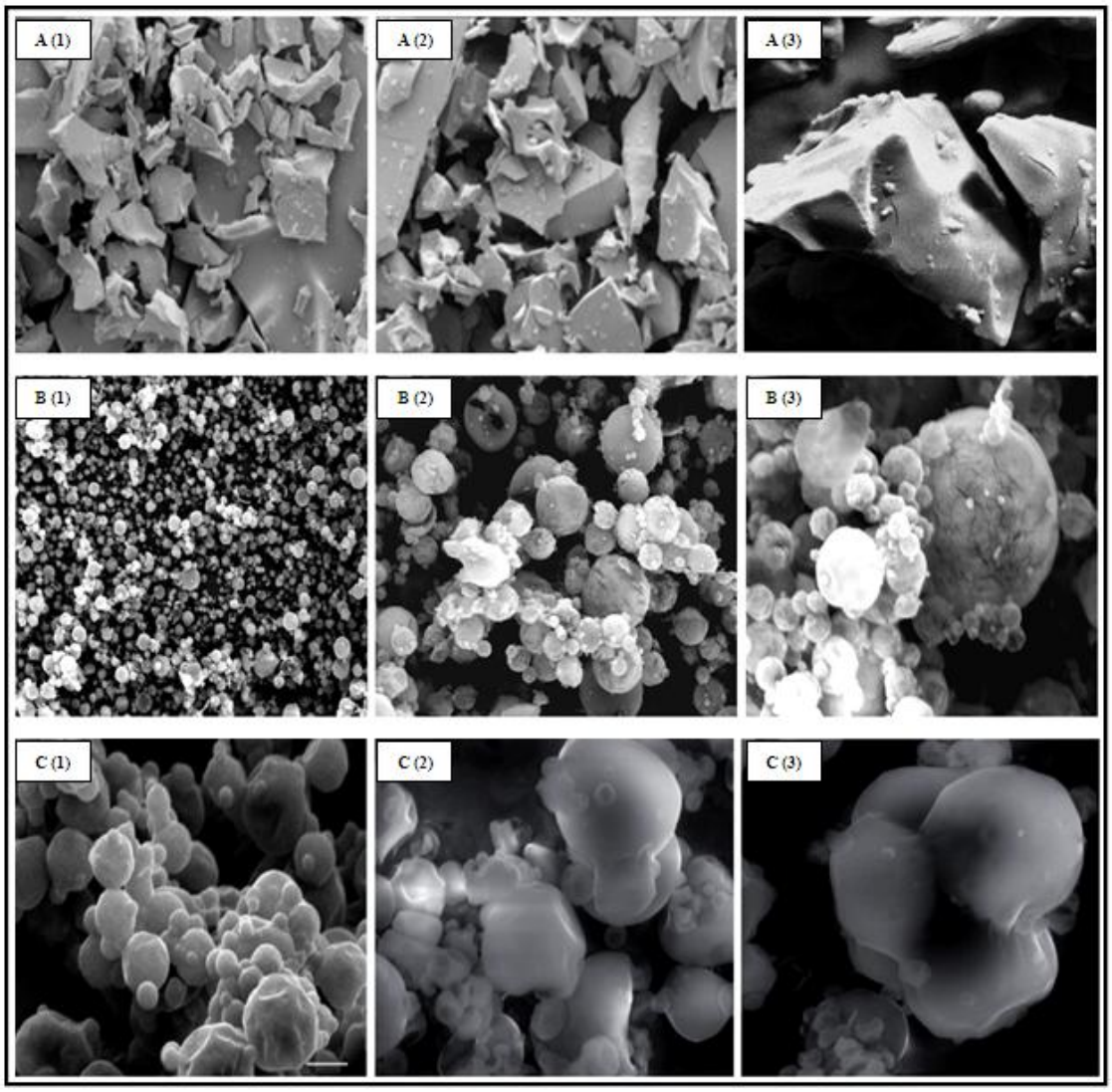

Fig. 10: Micrographs of particles of licorice powders obtained by different drying processes encapsulated in constant maltodextrin-Arabic gum mixed (2:1) in level 20 %. :
(A) Freeze drying process, at a magnification of : A (1) 300×; A (2) 1000×; A (3) 2500×.
(B) Spray drying process, at a magnification of : B (1) 300×; B (2) 1000×; B (3) 2500×.
(C) Vacuum drying process, at a magnification of : C (1) 300×; C (2) 1000×; C (3) 2500×.

BaP is a pro-mutagen which can be converted to the direct-acting mutagenic metabolite by cytochrome p450 isozymes, which are associated with BaP activation. Flavonoids inhibited the direct (nonactivated) mutagenicity of BaP in *Salmonella* strain TA98. Flavonoids can also inhibit the mutagenicity some of the cooking mutagens including BaP. Flavonoids inhibit the mutation or initiation caused by inhibition of activation of pro-mutagens and trap the electrophiles by chemical reaction or conjugation; antioxidant activity or scavenging of reactive oxygen species [65].

Eventually human dietary studies will be required to demonstrate that a co-exposure to cooking mutagens and flavonoids actually inhibits the activation and/or DNA binding of the mutagens. The effect of dietary flavonoids is that they can reduce the intestinal uptake of BaP, [12].

The previous studies have shown the antimutagenesis effect of glycyrrhizic acid (GA) [64]. In the presence of Aroclor 1254-induced rat liver S-9 as the enzyme source for the metabolic activation, β-GA was more effective than α-GA in inhibiting the mutagenicity induced by benzo[*a*]pyrene (BaP), and 2-aminofluorene in *Salmonella typhimurium* strains TA100 and TA98. At 0.5 μg/plate, β-GA and α-GA inhibited aflatoxin B_1-induced mutagenesis in the TA98 strain by 77% and 23%, respectively as shown by Wang *et al.,* [64]. Licorice extracts exerted a blocking effect on mutagenesis in strains of *S. typhimurium* (TA97, TA98, TA100, and TA102) induced by furapromidum, zhengdingmycin hydrochloride, *N*-

methyl-*Ń*-nitro-*N*-nitrosoguanidine (MNNG), and methylmethanesulfonate [50]. Licorice extract was found to protect against mutagenesis induced by cyclophosphamide in mice. Concomitantly, specific effects on hepatic microsomal drug metabolism and mutagen-DNA binding have been demonstrated [41].

Table 4: Antimutagenic activity of licorice powder extracts obtained by different drying processes against MNNG (0.1 μg, 0.01 μg/plate) on *Salmonella typhimurim* TA 98 and TA 100 under simulated gastric juice or intestinal juice.

Licorice (mg/plate)	Gastric juice				Intestinal juice			
	Revertants/plate[a]		Percent inhibition (%)		Revertants/plate		Percent inhibition (%)	
	TA98	TA100	TA98	TA100	TA98	TA100	TA98	TA100
Freeze Drying								
Control	229.2±6	273.3±7	0	0	239.2±4	252.6±3	0	0
1	135.4±8	189.5±8	40.9±0.8	30.7±0.5	131.1±5	172.5±9	45.2±1.3	31.7±2.2
2	122.6±3	170.4±11	46.5±0.7	37.8±0.9	103.5±6	122.4±12	56.7±0.7	51.5±1.4
3	106.1±10	97.8±14	53.7±1.2	64.2±1.3	92.3±11	91.6±9	61.4±0.6	63.7±0.4
Spray Drying								
Control	238.4±2[b]	269.6±3	0	0	366.6±10	298.3±14	0	0
1	145.4±3	133.3±3	39.0±1.5	50.6±1.1	218.6±6	135.5±4	40.4±0.6	54.6±0.8
2	120.2±7	115.1±10	49.6±1.9	57.3±1.2	167.4±11	119.7±7	54.3±0.8	59.9±0.7
3	89.7±4	91.3±5	62.7±0.7	66.2±0.9	109.8±9	76.1±8	70.1±1.9	74.5±1.3
Vacuum Drying								
Control	148.0±9	197.0±11	0	0	155.5±3	252.0±9	0	0
1	124.0±4	163.6±7	16.2±0.6	17.0±1.7	129.3±21	194.0±23	16.8±0.9	23.0±0.6
2	106.7±1	133.0±4	27.9±2.5	32.5±1.1	118.7±6	171.1±4	23.7±1.4	32.1±0.8
3	70.5±17	82.7±7	52.4±0.2	58.1±0.3	84.7±9	119.3±2	45.5±1.5	52.6±1.6

[a] Triplicate plates were tested per dose per experiment.
[b] Datas are presented as the mean ±S.D. of triplicate determinations.
MNNG: *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine.

Dietary inhibitors of mutagenesis and carcinogenesis are of particular interest, hence they may be useful for human cancer prevention on recently, several flavonoids and phenolic compounds have been demonstrated to have an anti-mutagenic effect on various mutagens or carcinogens [7]. Also dietary flavonoids, a group of polyphenols, suppressed the metabolism of the widespread food carcinogen 2-amino-3-methylimidazo-[4,5-*f*] quinoline (IQ) [60], in the presence of a hepatic activation system derived from Aroclor 1254-treated rats, is a typical heterocyclic amine mutagen found in cooked foods.

In the present study, a weak relation exists between the phenolic content of the licorice extract and the antimutagenic effect when using the indirect acting mutagens BaP and the direct acting mutagen MNNG. This study also suggested that licorice extracts are useful nutritional antioxidants and their supplementation nullifies oxidative stress and licorice extracts can serve as such electron acceptors. When the licorice extracts are incubated, the protective effect could be ascribed to a direct interaction of the activated mutagenic metabolite that diffuses from the bottom layer. Therefore the results of the present study show that the in vitro gastrointestinal model can be applied in mechanistic studies on antimutagenicity in food and in research on the development and efficacy of food products.

Conclusion:

The results obtained confirm that the drying technique has a strong impact on the powder quality in relation to the content of bioactive compounds. Therefore, drying parameters used should be carefully considered. In general, among drying techniques analyzed, spray drying is regarded to be the one having the smallest impact on the degradation of bioactive compounds, due to a short time and relatively soft conditions of the process. Spray drying, being the most common and cheapest (30–50 times cheaper than freeze drying) technique for the production of food materials, can be recommended for licorice powders production.

Morphological study revealed that the spray dried powder had small sized particles that were densely packed. Spray dried licorice powder made with 20% maltodextrin-Arabic gum mixed and processed at 140 °C inlet temperature had less hygroscopicity, a_w, a good quality licorice powder, acceptable color, TCC and TAA. In conclusion, drying techniques have an impact on selected quality parameters, and different drying techniques cause changes in the content of bioactives analyzed.

The licorice powder extracts showed antimutagenicity against BaP and MNNG in *Salmonella typhimurium* TA98 and TA100. Amounts of 3 mg per plate of licorice powder extracts were sufficient to inhibit the mutagenicity induced by any of the BaP (Benzo[*a*]pyrene) and MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) concentrations used. Licorice extracts has already demonstrated its effective antimutagenic activity. In digested licorice powder extracts obtained by different drying processes also showed antimutagenicity. In all cases, concentration dependent inhibition was observed. There were no significant differences in antimutagenicity on the digested licorice powder extracts. All different licorice powders obtained by different drying processes in our study contained antimutagens and the extracts showed similar levels of antimutagenic activity. The antimutagenic effects

of Licorice powder extracts depended on the mutagen and dose levels.

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