

Physicochemical properties of serish root (*Eremurus spectabilis*) fructan as affected by drying methods

A. Pourfarzad^{1*}, M.B. Habibi Najafi², M.H. Haddad Khodaparast² and M. Hassanzadeh Khayyat³

¹Department of Food Science and Technology, Faculty of Agricultural Sciences, University of Guilan, P.O. Box 41635-1314, Rasht, Iran; ²Department of Food Science and Technology, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran; ³Pharmaceutical Research Center, Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, Iran; amir.pourfarzad@gmail.com

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RESEARCH ARTICLE

Abstract

The fructans, inulin and oligofructose, are relatively unique functional food components because of their chemical nature and the combination of their physiological and nutritional effects that affect gastrointestinal functions. In the present study, the physicochemical properties of dried serish root fructans prepared by application of freeze drying, spray drying, vacuum drying and oven drying at 80 and 105 °C were evaluated. The oven-dried and freeze-dried fructans seem to be more hygroscopic than the spray and vacuum oven-dried fructans. The oven-dried sample at 105 °C showed the least water solubility among all samples. Fructan contents, purities, and the degree of polymerisation for dried serish fructan samples were 85-87, 79-80 and 13-14%, respectively. The drying method affected the microstructure of the fructan particles leading to differences in their physical properties (particle size, shape, zeta potential, colour and solubility). The colour analysis suggested that the successive combination of spray drying and freeze drying methods had the least negative effect on appearance among the drying treatments. As evidenced by the X-ray diffractograms, the oven and spray-dried samples exhibited amorphous structures, while the freeze and vacuum drying of serish fructans tend to produce semi-crystalline dried products. Differential scanning calorimetry showed that Freeze-drying gave the highest onset and mid-point melting temperatures, whereas oven and spray drying showed the lower melting enthalpy than the freeze dried and vacuum dried samples. FT-IR spectral analysis showed no major structural differences in fructan samples produced by various drying treatments.

Keywords: freeze drying, fructan, oven drying, serish root, spray drying, vacuum drying

1. Introduction

Fructose is a six-carbon monosaccharide that occurs widely in plant foods in a variety of forms including the free monosaccharide, combined with glucose to form the disaccharide sucrose, or polymerised to form fructans. Fructans are oligo- and polysaccharides consisting of short chains of fructose units with a single D-glucosyl unit at the nonreducing end (Roberfroid and Delzenne, 1998; Roberfroid, 2005). The degree of polymerisation (DP) varies from two to several hundreds, with the major components of fructans being inulin (mostly DP 2-60) and oligofructose (DP 2-10) (Prosky and Hoebregs, 1999). Fructans are considered important prebiotics. In addition

to its interesting nutritional and health benefit properties, fructans are also used in food formulations for its techno-functional properties such as fat substitution, bulking ability and water retention, etc. (Blecker *et al.*, 2001; Botrel *et al.*, 2014; Dogan and Tokar, 2015; Moghari *et al.*, 2015; O'Brien *et al.*, 2003).

Serish (*Eremurus spectabilis*) belongs to the family of Liliaceae and is geographically distributed in the area of South Asia and Central Asia, including Iran, West Pakistan, Afghanistan, Iraq, Turkey, Palestine, Lebanon, Syria and the Caucasus. Its roots accumulate high levels of fructans during their growth. This root has traditionally been used to cure of jaundice, liver disorders, stomach irritation,

pimples and bone fractures. In addition, it has been used as glue for industrial purposes (Brayan, 1989; Brickell, 1996; Crockett, 1972; Dashti *et al.*, 2005).

The production of standardised dried extracts from plants is an important subject for herbal processing industries in order to guarantee a consistent and appropriate level of bioactive substances, as well as the product's safety, efficacy, and quality (Oliveira *et al.*, 2012). However, the attainment of optimum processing conditions is a challenging task due to the complex mixture of substances present in an herbal extract, which are associated with other factors that can affect the drying operation and product quality (Shene *et al.*, 2003). The functional properties of fructans have direct influence on the scope of their application and commercial values, which are claimed to be significantly affected by the drying processes. Various processes are recommended for dehydration of fructan extracts. The method of choice is selected based on the physical and chemical properties, thermal stability, and the intended use of the product (Cortes-Rojas and Oliveira, 2012).

To the best of our knowledge, there are no reports on the effects of drying process on the functional properties of serish fructans. Thus, the present study is considered the first attempt aiming to examine the effects of different drying methods (which include spray drying, freeze drying, vacuum drying and oven drying at two temperatures 80 and 105 °C) on fructan content, purity, degree of polymerisation, conductivity, zeta potential and colour of the dried serish fructans. This study will help understanding the relationship between drying processes and properties obtained from scanning electron microscopy, X-ray diffraction (XRD), Fourier-transform infrared (FT-IR) spectroscopy and differential scanning calorimetry in order to provide useful information regarding their application in food industry.

2. Materials and methods

Preparation of serish roots powder

The serish root powder (6.2% moisture content) prepared from batch of roots which were collected from Neishabour region (Khorasan, Iran) was purchased from the local medicinal plant market, Mashhad, Iran. Samples were passed through a 50 µm sieve and stored in a dry container for further use.

Preparation of fructan concentrate

Fructan extraction was carried out in a water bath (model WB/0B7-45; Memmert Company, Schwabach, Germany). Serish root powder was suspended in distilled water at a ratio of 1:50 (w/v) and was allowed to stand at 85-90 °C for 30 min (Pourfarzad *et al.*, 2015). The suspension was then filtered through muslin cloth to remove the insoluble

residues. The resulting solution was turbid due to the presence of particulate and colloidal matter, i.e. pectin, protein, and cell wall substances (Hansen and Madsen, 1992). To remove these impurities, the preparation was mixed with 5% slurry of calcium hydroxide at 50-60 °C for 30 min, resulting in the formation of a flocculent precipitate and a brighter yellow supernatant. By this technique, the pH of solution rose from 5-6 to 10-12. After filtration under vacuum using paper filter (Whatman no. 4; Whatman International Ltd., Maidstone, UK), 10% phosphoric acid was added to the filtrate with vigorous stirring to adjust its pH to 8-9, causing the precipitation of surplus calcium and coagulation of organic material. The mixture was permitted to stand at 60 °C for 2-3 h before re-filtration (Whatman no. 4; Whatman International Ltd.). The clarification process was repeated twice. Activated carbon powder (150 µm) was then added to the filtrates at 60 °C and mixed for 15-30 min to facilitate elimination of coloured materials. The treated solution was filtered (Whatman no. 1; Whatman International Ltd.) and the clear achieved solution was further concentrated by rotary evaporation at 70 °C (Paseephol *et al.*, 2007). The concentrated inulin solution with soluble solids level of 40 °Brix which was obtained after the evaporation process, was mixed with 8.5 parts by weight of ethanol (abs. 99%) and stored at 20-25 °C for 3 days (Pourfarzad *et al.*, 2014). After storage, the supernatant was removed and the precipitate washed with ethanol. The precipitate was then taken to undergo the different drying methods.

Drying methods

The drying methods reported previously by Wang *et al.* (2010) were used in this study. The fructan precipitate was spray dried using an Eyela spray dryer (Eyela SD-1; Eyela, Tokyo, Japan) equipped with a 0.4 mm two-fluid nozzle. The inlet and outlet air temperatures were 120 and 80 °C, respectively. During the experiments, the blower flow meter, air pump (pressure) gauge and feed spray rate were set at 0.5 Nm³/min, 1 kg/cm² and 200 ml/h, respectively. The product was collected in a cyclone separator (Kim *et al.*, 2000).

Precipitated fructan of serish was pre-frozen at -20 °C for 24 h prior to freeze-drying. The pre-frozen sample was then transferred to the freeze drier chamber (Martin Christ 8891, type 317; Martin Christ; Osterode, Germany) and held for 24 h. The vacuum pressure of the dryer was set at 20 Pa, the plate temperature was 20 °C, and the condenser was at -30 °C. The freeze dryer was equipped with a gradient temperature controller.

Precipitated fructan of serish was vacuum dried in a vacuum drier (OT 53, Micro Precision Calibration Inc., Grass Valley, CA, USA) for 24 h. The vacuum and temperature were maintained at 60 Pa and 60 °C, respectively.

Precipitated fructan of serish was dried in a hot air oven (model 100-800; Memmert, Schwabach, Germany) at two drying conditions: 105 °C for 8 h, and 80 °C for 24 h.

All dried fructans were milled and passed through a 1.0 mm mesh sieve and packed in the air-tight containers prior to the analysis.

Determination of moisture content

Moisture content (dry basis) was determined by drying the samples in an oven (model 100-800; Memmert) at 105 °C for 24 h (AOAC, 1995).

Determination of total carbohydrate

Total carbohydrate was assayed colorimetrically using the phenol-sulphuric acid method (Paseephol *et al.*, 2007). The dried sample was mixed with 1 ml distilled water, 1 ml of 5% phenol and 5 ml of sulphuric acid. Sample weights were adjusted to obtain a reading of 10-70 µg. The mixture was incubated in a water bath at 30 °C for 20 min. The solution appeared as a yellow-orange colour and its absorbance was measured at 490 nm using a UV-visible spectrophotometer (model S2000; WPA Lightwave, Saffron Walden, UK). A series of D(-)-fructose ($M_w=180.16$; Fluka, Buchs, Switzerland) solutions of known concentration were used to plot a standard curve.

Determination of reducing sugar

The concentrations of soluble reducing sugars were measured using a 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). After drying, the sample was mixed with distilled water 10 times, and 3 ml of the diluted sample was transferred to a test tube where it was mixed with 3 ml of the DNS reagent. Sample weights were adjusted to obtain a reading of 10-500 µg. After mixing it with a vortex, samples were heated in water bath at 90 °C for 10 min followed by rapid cooling to ambient temperature. The absorbance of all samples was measured at 575 nm using a UV-visible spectrophotometer. The calibration curve was assembled using D(-)-fructose as standard.

Determination of fructan content

The total fructan content was conducted according to the method described by Lingyun *et al.* (2007) and Paseephol *et al.* (2007) and was measured by the difference between total carbohydrate and reducing sugars.

Purity determination

The purity value was calculated as follows (Lingyun *et al.*, 2007; Paseephol *et al.*, 2007):

$$\text{Purity (\%)} = \frac{\text{fructan content of dried sample}}{\text{dry matter content of precipitate}} \times 100 \quad (1)$$

The dry matter content of precipitate was determined gravimetrically (AOAC, 1995).

Degree of polymerisation

The average chain length, as an index of degree of polymerisation, was calculated according to Paseephol *et al.* (2007):

$$\text{Degree of polymerisation} = \frac{\text{total carbohydrate}}{\text{total reducing sugar}} \quad (2)$$

Water solubility

Solubility of the dried products was determined according to Cortes-Rojas *et al.* (2012). One hundred milligrams of powder was weighed exactly and 10 ml of water was added to the sample followed by vigorous mixing for 10 min on a magnetic stirrer. The samples were then centrifuged for 5 min at 3,000×g in a laboratory centrifuge (model 4-16KS; Sigma, Osterede, Germany). An aliquot of approximately 2 ml of the supernatant was transferred to a previously weighted Petri dish. The solid concentration was determined by the oven drying method previously described. Results were expressed as grams of soluble powder per 100 g of water.

Particle size

The dried samples using various methods were redissolved in deionised water. Solution (0.09%, w/w) was prepared by stirring the solids at 25 °C for 30 min. The particle size distribution of the different fructan solutions was determined by laser scattering using particle size analyser (VASCO 3; Cordouan Technologies, Pessac, France). A sufficient amount of fructan dispersion was introduced in the sample cell of the particle size analyser under moderate stirring. The refractive index used was 1.33. Measurements were performed at room temperature in triplicate. The average particle size, expressed in nm, refers to the median diameter in volume.

Zeta potential and conductivity

Zeta-potential of fructan solutions (0.09%, w/w) was deduced at pH 5.5 and 25 °C using a laser zetameter (ZetaCompact Z8000; CAD Instrumentation, Les Essarts Le Roi, France) under a 7 V/cm electric field. Results were based on an automated video analysis of about 100 particles. An electric field of 7 V/cm was applied. The conductivity of samples was also measured at the same time.

Colour analysis

The colour of the powders was evaluated using a colorimeter (Chroma meter CR-410; Sensing, Inc., Tokyo, Japan). The colour was expressed in terms of lightness (L^*), redness (a^*), and yellowness (b^*).

Appearance and morphology

The morphology of samples was observed using scanning electron microscope (Muir *et al.*, 2007). For that, a small amount of finely ground material was scattered evenly onto the surface of an aluminium stub covered with a 12 mm diameter carbon tab and sputter-coated with gold film in a sputter coater (SC7620; Polaron Sputter Coater, Quorum Technologies, Newhaven, UK). Observations were made using the scanning electron microscope (LEO 1450 VP; Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 20 kV. All the samples were examined at magnifications of 200 $\times$ and 500 $\times$.

X-ray diffraction

Samples were analysed for crystalline structures using a procedure previously described elsewhere (Ronkart *et al.*, 2007). The apparatus used was a Unisantis XMD-300 (Unisantis, Georgsmarienhütte, Germany) with a nickel-filtered $\text{CuK}\alpha$ radiation ($\lambda=1.54178 \text{ \AA}$) generated by an anode device operating at 40 kV and 0.8 mA in conjunction with a proportional detector. The patterns were recorded with a fixed time of 0.4 s per step of 0.02° in the $30<2\theta<40^\circ$ range at 20°C .

Fourier-transform infrared spectroscopic analysis

FT-IR spectroscopic studies were performed in transmission mode on a spectrophotometer (Paragon 1000; Perkin Elmer, Waltham, MA, USA). Samples were blended with KBr powder, and pressed to form a tablets before measurement. This method was chosen for its express execution and qualitative information for certain functional groups. Spectra were obtained at 4 cm^{-1} of resolution from 4,000 to 400 cm^{-1} . The interference of water and CO_2 from air was deducted during scanning.

Differential scanning calorimetry

The thermal properties of fructan samples were performed using differential scanning calorimetry with a differential scanning calorimetry (DSC; Mettler Toledo, Greifensee, Switzerland). The DSC curves were obtained during heating of the samples from 10 to 300°C with heating speed interval of $5^\circ\text{C}/\text{min}$. All these DSC experiments were made using unhermetic aluminium pans of matched mass, i.e. the empty sample and reference pans were of equal mass to within $\pm 0.10 \text{ mg}$. The analysed sample mass was 2 mg. Dry

nitrogen was used for purging the DSC cell. Temperature and enthalpy calibrations were carried out using indium (Panchev *et al.*, 2011). Melting temperatures of onset and mid-point melting transition and change in enthalpy at melting were obtained from DSC curves.

Statistical analyses

Results were reported as the average of three replications (all treatments were evaluated in three batches). In order to assess significant differences among different drying methods, a complete randomised design of triplicate analyses of samples was performed using the Minitab 15 (Minitab Inc., State College, PA, USA) software. Duncan's new multiple range tests were used to study the statistical differences of the means with 95% confidence.

3. Results and discussion

Moisture content and solubility of dried samples

The quality of any powder as food additive is based on a variety of properties that depend on its ultimate application. In general, the final moisture content and solubility are of primary importance (Souza *et al.*, 2009). Moisture content is a parameter required for the activation of enzymes and the potential proliferation of microorganisms. It is a critical parameter affecting its shelf life during the storage. The effect of different drying techniques on the moisture content of serish fructans was shown in Table 1. The results indicated that the moisture content of serish fructans was partly influenced by the method of drying. In fact, production of dried fructans by different methods led to some difference on the moisture content. This could be due to different drying conditions (i.e. time and temperature). The results indicated that the moisture content of differently dried fructans ranged from 4.53 to 8.06%. In the present work, especially 105°C oven dried samples had the lowest moisture content. Therefore, longer shelf life is expected for this sample than the other dried samples, due to its lower moisture content. The powder with lower moisture content is more hygroscopic, which is related to the greater water concentration gradient between the powder and the surrounding air (Tonon *et al.*, 2008). In the current study, the oven (80 and 105°C) and freeze-dried fructans seem to be more hygroscopic than the spray and vacuum dried samples.

Solubility is an important property of powders and is directly related to drying method and composition of dried sample. The freeze and oven-dried (105°C) samples showed the most and least water solubility among all samples (Table 1). This is most likely due to the lowest particle size of freeze dried samples and transformation of the fructan structure caused by drying at the excessive temperature (105°C). In fact, oven drying (105°C) may result in the thermal degradation of the fructan structure. Although

Table 1. Physicochemical properties of serish fructan prepared by different drying methods.¹

Dried sample	Spray	Freeze	Vacuum	80 °C oven	105 °C oven	SEM ²
Moisture content (%)	8.06 ^a	6.55 ^{ab}	8.16 ^a	5.89 ^{ab}	4.53 ^b	-0.336
Solubility (%)	66.62 ^b	71.32 ^a	60.70 ^c	55.53 ^d	40.70 ^e	-0.013
Fructan content (%)	85.65 ^a	86.07 ^a	85.38 ^a	85.23 ^a	86.51 ^a	-0.242
Purity (%)	79.34 ^a	79.74 ^a	79.09 ^a	78.96 ^a	80.14 ^a	-0.224
Degree of polymerisation	12.83 ^a	13.08 ^a	14.26 ^a	12.65 ^a	13.35 ^a	-0.493
Particle size (nm)	405.56 ^c	358.60 ^c	685.59 ^a	524.67 ^b	656.29 ^a	-14.66
Zeta potential (mV)	-24.11 ^d	-30.67 ^e	-15.60 ^a	-18.77 ^c	-17.06 ^b	-0.068
Conductivity (mS/cm)	0.018 ^a	0.017 ^a	0.017 ^a	0.018 ^a	0.017 ^a	-0.0003
L* ³	71.20 ^a	59.22 ^b	35.12 ^e	48.15 ^c	37.41 ^d	-0.116
a* ³	1.08 ^d	0.68 ^e	1.96 ^b	4.06 ^a	1.67 ^c	-0.02
b* ³	7.23 ^d	5.45 ^e	7.45 ^c	11.13 ^a	8.05 ^b	-0.01
T _i (°C) ⁴	119.94 ^d	168.49 ^a	151.38 ^b	122.55 ^{cd}	124.02 ^c	-0.394
T _m (°C) ⁴	152.00 ^d	181.15 ^a	171.58 ^b	163.29 ^c	146.16 ^e	-0.227
ΔH (J/g) ⁴	41.10 ^a	92.86 ^c	53.44 ^b	41.33 ^a	40.30 ^a	-0.223

¹ Each observation is a mean of three replicate experiments (n=3); values in rows with different letters are significantly different ($P \leq 0.05$).

² SEM = standard error of the mean.

³ L* = lightness; a* = redness; b* = yellowness.

⁴ T_i, T_m and ΔH represent the onset and mid-point melting temperature and change in enthalpy at melting, respectively.

oven drying is a low cost processing method as compared to spray drying or freeze drying, the present study reveals that it provides serish fructan with lower quality than freeze drying and spray drying in terms of water solubility and hygroscopic properties.

Chemical properties of dried samples

The fructan content, purity and the degree of polymerisation of fructans produced by various drying methods are presented in Table 1. The fructan content, purities, and the degree of polymerisations for dried samples ranged between 85.23-86.51%, 79.09-80.14% and 12.65-14.26, respectively. No significant difference was observed for fructan contents of dry samples between drying methods. In addition, drying methods seem to have no significant effect on the purity and the degree of polymerisation of the samples. The purities in the fructan samples produced by various drying methods were near to that of commercial fructans, which is above 80%. The low degree of polymerisation values for fructans produced by various drying methods may be either due to the source of the fructan (serish) or to the hydrolysis occurred during extraction process.

Particle size, zeta potential and conductivity

Generally, the particle size is one of the critical physical properties of powders that affect zeta potential, colour, solubility, density and sensory mouthfeel (Wang and Johnson, 2001). In order to explain the differences

between the dried fructan samples, their particle sizes were determined (Table 1). These data indicated that the freeze-dried fructans had the smallest particle size among others. This is because the formation of ice crystal in the material during freeze drying helps providing more porous structure than the other fructan powders. Therefore, the freeze-dried fructans had the smallest particle size among the samples. The relatively bigger particle size of spray dried than the freeze dried samples may be due to the fact that particles are agglomerates of smaller separate particles brought about in the drying steps that was present, as is shown in the scanning electron micrographs images. The bigger particle size of oven and vacuum dried samples could be due to the lower porosity and tighter surface that lead to unsuccessful milling and subsequently higher particle size than the freeze and spray dried samples.

By measuring the zeta-potential, it is possible to probe a characteristic colloidal property in a complex mixture of particles, making it a useful technique to explain their behaviour. As shown in Table 1, the zeta potential and conductivity of fructan solutions varied from -15.60 to -30.67 mV and from 0.017 to 0.018 mS/cm, respectively. The zeta potential can be used as a measure of the electrostatic repulsive forces, which can give an indication of the potential stability of the solutions containing fructan particles. High absolute values of zeta potential mean better stability because of the mutual repulsion between the electrical double layers of macromolecules (Acedo-Carrillo *et al.*, 2006). On the other hand, when the solutions have

low absolute zeta potential value then there is no force to prevent the molecules coming together. The dividing line between stable and unstable dispersions is generally taken at either +30 or -30 mV. Solutions with zeta potentials higher than +30 mV or lower than -30 mV are normally believed to be stable (Sherman, 1970). As indicated in Table 1, the lowest zeta potential (-30.67) belongs to the freeze dried sample. All dried samples, except the freeze-dried sample, have zeta potential higher than -30 mV, which means that their solutions will have slightly higher instability compared to the freeze-dried sample. Note that the specific area of the freeze-dried and spray dried samples is larger than others because of the smaller size of the particles. The high specific area of these particles may also facilitate a higher level of lipid and protein adsorption in the food and drug environments and the strongest negative zeta potential. Furthermore, it is known that the magnitude of the zeta potential is indirectly proportional to the particle diameter (Rowlands and O'Brien, 1995). Smaller particle sizes would thus be expected to yield zeta potentials that are bigger in magnitude, as was indeed found for freeze and spray dried preparations.

On the other hand, no significant difference was found on conductivity of samples obtained from drying methods performed in this study ($P>0.05$) confirming insignificant difference between moisture content of them. Also, this result indicated that there was not any inorganic impurity during drying process. The conductivity of foods has been found to be increased with presence of water and ions (Barbosa-Canovas *et al.*, 2006).

Colour analysis

The colour differences in serish fructan powders prepared by different drying methods are presented in Table 1. There were significant differences between the colour values of the serish fructan samples produced by various drying methods. Ideally, the serish fructan should be colourless in order to avoid any colour changes in food products upon its addition. The spray dried sample had the highest value for lightness (L^*) among the samples tested in this study. This is because the spray drying has very short residence time and the powder particles are subjected to drying condition for a very short time and also the lowest particle size of it compared to other drying methods (Wang *et al.*, 2010). Whereas, oven dried sample (80 °C) had the highest redness (a^*) and yellowness (b^*) which can be attributed to the thermal degradation caused by considerably long drying time at high temperature. The lower hunter colour values of the 105 °C oven dried sample than the 80 °C oven dried sample can be attributed to more caramelisation and colour degradation via higher drying temperature. As a result, the oven dried (80 °C) powder appeared dark and red compared to other samples. Consequently, its application would be restricted to limited products such as sausages.

Fructan produced by vacuum drying had the lowest value for lightness; while freeze dried sample had the lowest redness and yellowness among the samples produced by various drying methods. The hunter colour values suggested that the spray drying as well as freeze drying methods had the least negative effect on appearance among the drying treatments tested in this study. Depending on the food and drug applications, a drying method may be chosen to obtain fructan with desired appearance. These results are in agreement with findings of other studies on flaxseed gum powders, soy hull pectin and strawberry puree (Monsoor, 2005; Wang *et al.*, 2010).

Appearance and morphology

Scanning electron micrographic studies of serish fructan powders obtained by different drying processes are shown in Figure 1. In this process, round and lamellar particles are formed, as can be seen in the scanning electron photomicrographs. Most of the spray-dried particles have spherical or oval shape and smooth surface particles, because this process does not involve product friction inside the drying chamber and also results in a small particle diameter (Figure 1A). The smooth sides of a single particle are indicating more flowability and less susceptibility to oxidation because of lesser surface area. Other studies performed on spray drying of inulin solutions are also indicated the formation of spherical surfaces (Toneli *et al.*, 2010). Ronkart *et al.* (2007) studied the effect of drying air temperature on the microstructure of powdered inulin and observed that the inulin samples that were dried at 120 and 170 °C were intact whereas at 230 °C, the surfaces were damaged. Freeze-dried powder (Figure 1B), showed a skeletal-like structure and was more porous than the other fructan powders. The reason is because the ice formation in the material during freeze drying helps avoid shrinkage and collapse of the structure and shape resulting in an insignificant modifying in volume (Ratti, 2001). The microstructure of vacuum and oven dried fructan powders (Figure 1C to 1E) were compact and exhibited irregular particles with sharp edges and considerable indentation as a result of crushing into powder. Vendruscolo *et al.* (2009) reported similar structure for vacuum oven dried polysaccharides of *Mimosa scabrella*. These results confirmed the higher solubility obtained for freeze and spray-dried samples compared to the other powder products.

X-ray diffraction

XRD is a common technique used to confirm the crystalline-amorphous state of dried products in a powder form. In general, crystalline material shows a series of sharp peaks, while amorphous product produces a broad background pattern. The presence of diffuse and large peaks in XRD patterns for samples containing amorphous

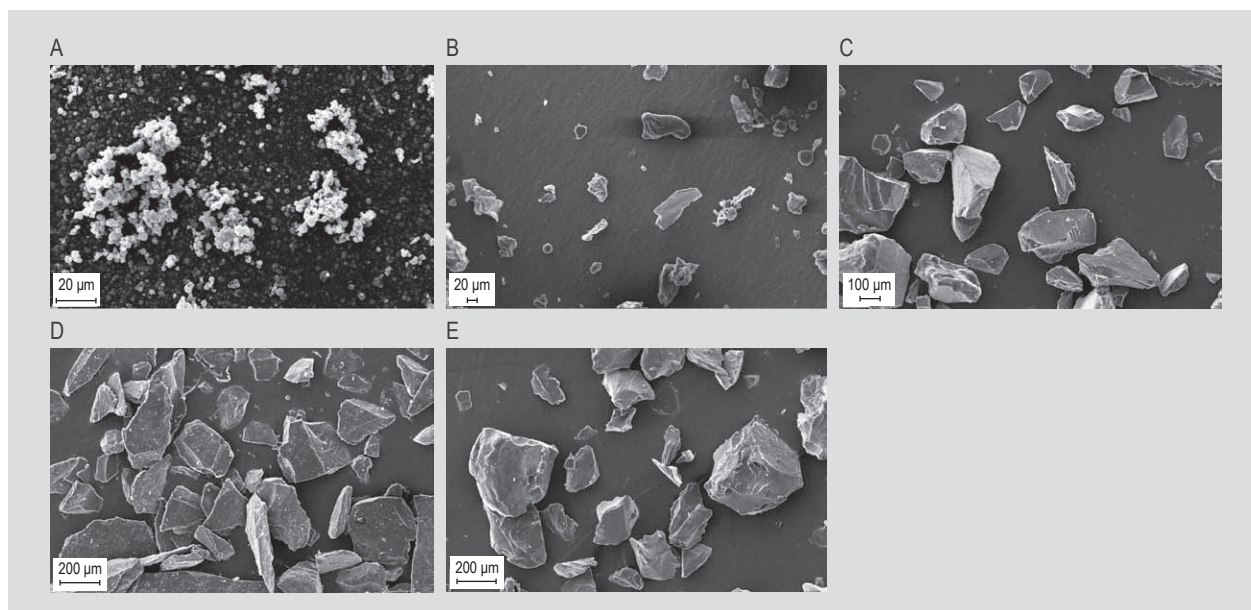


Figure 1. Scanning electron micrographs of (A) fructan powders dried using spray drying, (B) freeze drying, (C) vacuum drying, (D) oven drying at 80 °C drying, and (E) oven drying at 105 °C drying with magnification of 200 $\times$.

material is due to the fact that in the amorphous state, the molecules are disorderly displayed producing diffuse bands whereas crystalline materials yield sharp and defined peaks since they are presented in a highly ordered state (Caparino *et al.*, 2012). This tends to ensure a high level of purity and stability, particularly if the crystal is in its most thermodynamically stable form. In general, whenever amorphous material is present there must be significant concern since, relative to the crystalline state, the amorphous state is less thermodynamically stable.

Consequently, lower crystallinity of molecules results in the exhibition of greater chemical instability, enhanced dissolution rates and altered mechanical properties (Ahlneck and Zograf, 1990).

Figure 2 presents the XRD profiles of the dried fructan samples. According to the profile diffractogram, the systems presented amorphous and partially crystalline materials, i.e. peaks with considerable noises (spray and oven dried samples) and semi-defined peaks (freeze and vacuum

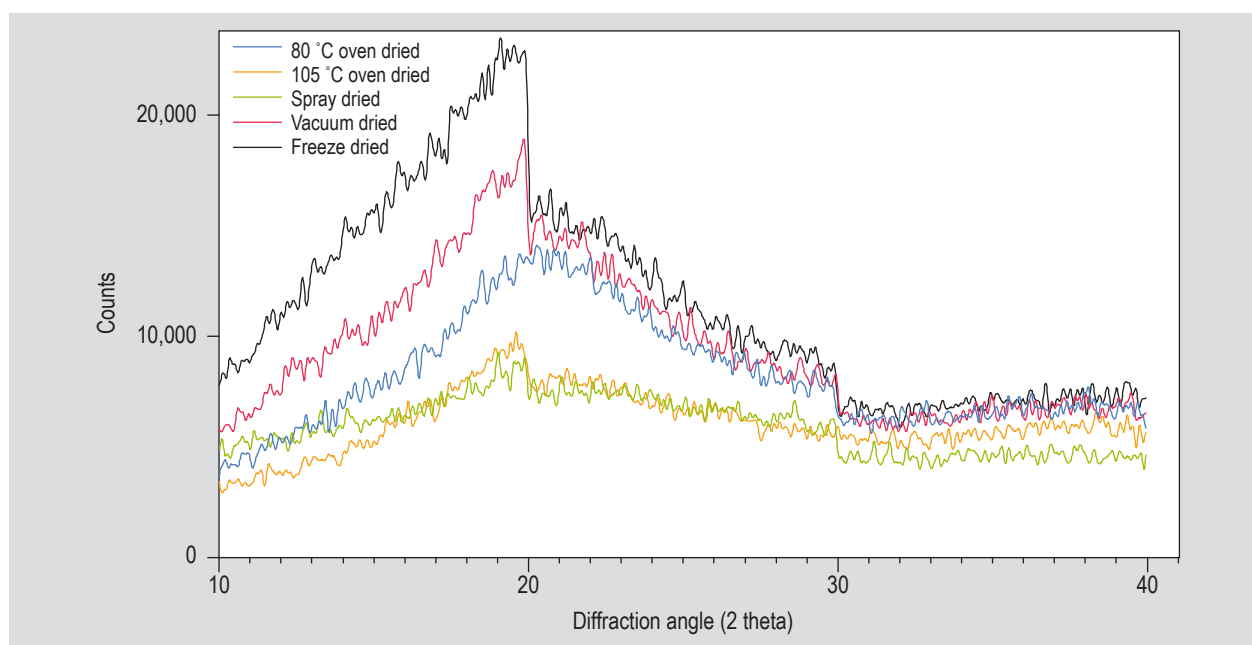


Figure 2. X-ray diffraction pattern of serish fructans prepared by different drying methods.

dried samples), which means that type of drying method had influence on the formation of partially crystalline structures. The presence of amorphous material may be due to the fact that during drying, the material did not reach the conditions necessary for crystallisation to occur. The absence of crystalline state observed in the spray-dried samples might be due to the characteristics of the drying process, which occurs rapidly, without sufficient time for the molecules to arrange in the so-called crystalline nets (Cano-Chauca *et al.*, 2005). On the other hand, the XRD pattern of oven-dried samples (especially at 105 °C) showed almost 0% crystallinity, which was probably due to the structural damage during drying. Furthermore, the freeze and vacuum drying of serish fructans tend to produce semi-crystalline dried products. The semi-crystalline component was characterised by the presence of relatively sharp reflections and wide diffusion maxima. The former was the result of coherent scattering from crystalline structure and the latter was originated from incoherent scattering caused by amorphous materials (Zhou *et al.*, 2009). The highest degree of crystallinity and subsequently the most stability of freeze dried sample among dried fructans could be explained according to the mechanism of freeze drying: the precipitation and crystallisation in-between different ice crystals induced by the supersaturation (Tang and Pikal, 2004). The influence of drying techniques on XRD patterns obtained in this study was similar to that observed for inulin (Ronkart *et al.*, 2007), mango powder (Cano-Chauca *et al.*, 2005; Caparino *et al.*, 2012) and lactose (Chiou *et al.*, 2008).

Fourier-transform infrared analysis

The infrared spectroscopy is a fast and convenient method for the investigation of functional groups of polysaccharides (Cakić *et al.*, 2002). The FT-IR spectra of fructans produced

by different drying methods are shown in Figure 3. The spectra are basically similar to the previously analysed spectra of other examined systems such as colloidal iron-inulin complex (Nikolic and Cakic, 2007) and fructans (Grube *et al.*, 2002). The absorbance around 600-700 cm^{-1} reflected the absorption of the C-H bending. The region between 1000 and 1,320 cm^{-1} corresponds to the stretching vibrations of (C-OH) side groups, the (C-O-C) glycosidic bond, ether (R-O-R), cyclic (C-C) and C-H (CH_3 group) bond vibrations (Shi *et al.*, 2012). The relatively strong absorption peak at around 1,611 cm^{-1} reflected the absorption of the C=O stretching vibration in the ring structure of fructan molecules (Zhao *et al.*, 2011). Some weak absorption peaks of about 3,250 cm^{-1} is believed to be a result of intermolecular H-bonded and O-H stretching modes, were observed in the spectra of samples (Nikolic and Cakic, 2007). In the IR area of about 3,400 cm^{-1} , there is a wide intense band. This band can be assigned to the O-H stretching vibrations of $\text{CH}_2\text{-OH}$ groups from a fructo-furanose unit (Zhao *et al.*, 2011). The absorption peaks in the spectra of samples showed some minor differences. The intensity of peaks around 600, 1,600 and 3,400 cm^{-1} was relatively higher in the spectra of 105 °C oven-dried sample. The enhancement of intensity at this wavenumbers may be related to relatively higher purity of this sample. In conclusion, FT-IR structure analysis shows that the drying methods had not any detrimental effect on the structure of serish fructan.

Differential scanning calorimetry

The melting temperature and enthalpy values of serish fructans prepared by different drying methods are illustrated in Table 1. Freeze drying gave the significantly highest ($P<0.05$) onset and mid-point melting temperatures.

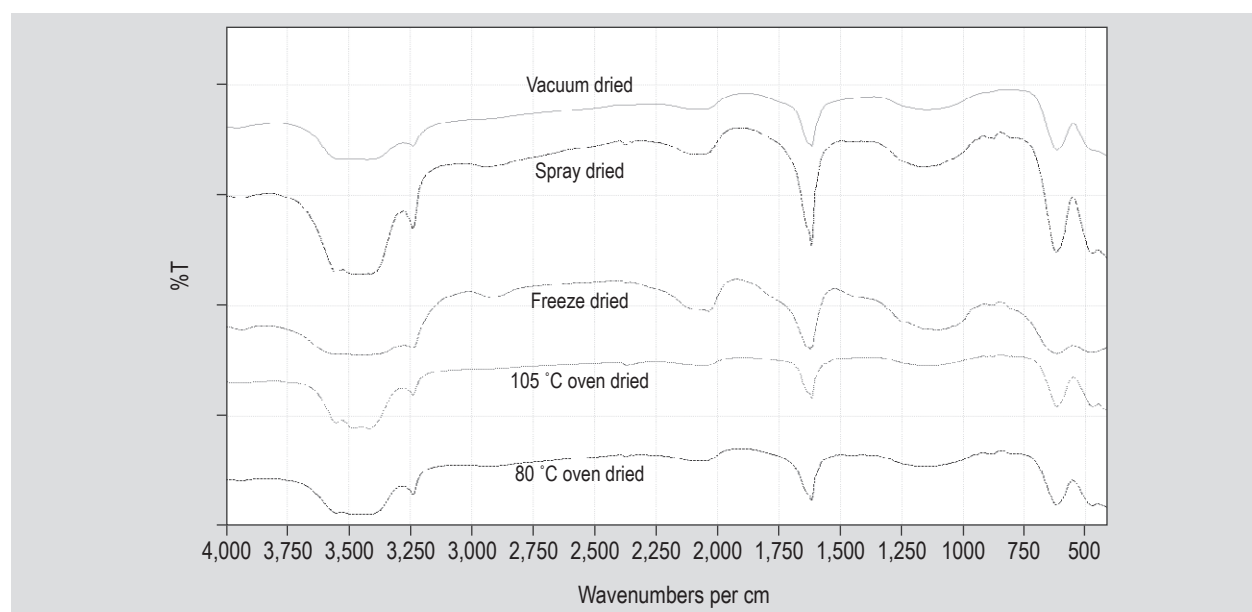


Figure 3. Fourier-transform infrared spectra of serish fructans prepared by different drying methods.

While oven drying at 105 °C showed the lowest peak melting temperature values when compared to other drying methods. The reason for such behaviour might be due to the higher crystallinity of freeze and vacuum dried samples than the other methods. As it is shown, freeze dried samples had the highest change in enthalpy at the endothermic transition taking place during melting of crystals. However, oven and spray dried methods had the lowest enthalpy. It is important to note that the crystalline phase acts as physical cross-links that increase the enthalpy (Tant and Wilkes, 1981). These results confirmed the highest crystallinity obtained for freeze-dried samples among fructan powder products.

4. Conclusions

In the current work, the effect of different drying processes on the physicochemical properties behaviour of serish root fructan was investigated. There were no significant differences ($P>0.05$) between dried powders in terms of fructan content, purity, the degree of polymerisation and conductivity. The freeze and oven-dried (105 °C) samples showed the most and least water solubility among all the samples, respectively. The lowest zeta potential (-30.67) belongs to the freeze dried sample. The hunter colour values showed that the successive combination of spray and freeze drying methods had the highest values for lightness among the samples; while, the oven dried (80 °C) powder appeared dark and red compared to other samples. Most of the spray-dried particles have spherical or oval shape and smooth surfaces. However, freeze-dried powder showed a skeletal-like structure. On the other hand, the microstructure of vacuum and oven dried fructan powders were compact and exhibited irregular particles with sharp edges and considerable indentation. As evidenced by the X-ray diffractograms, the oven and spray-dried samples exhibited amorphous structures, while the freeze and vacuum drying of serish fructans tend to produce semi-crystalline dried products. FT-IR spectral analysis showed no major structural differences in fructan samples produced by various drying treatments. Freeze-drying gave the highest onset and mid-point melting temperatures. While oven and spray drying showed the lower melting enthalpy than the freeze dried and vacuum dried samples. Overall, our study concludes that the freeze-drying method can produce superior quality serish fructan powder compared to other drying methods. The study provides an opportunity to the powder processing industry in selecting a better drying technique that can be applied for the manufacture of high quality fructan powder. Although, the oven drying is a low cost drying technique as compared to spray drying or freeze drying, the present study reveals that the application of oven drying method results in the weak physicochemical behaviour of fructan.

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