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INFLUENCE OF STORAGE TIME ON QUALITY OF SPRAY-DRIED EXTRACTS OF BASIL (*OCIMUM BASILICUM* L.)

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Summary

Sweet basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family, is the major essential oil crop which is cultivated commercially in many countries. The aromatic leaves of basil are used fresh or dried as a flavoring agent for foods, confectionery products and beverages. In the current study macerates of basil were spray dried with addition of 0%, 10%, 20% and 30% maltodextrin. In order to evaluate influence of storage time on quality of four basil powders the moisture content, rehydration, bulk density, total phenolic and total flavonoid contents were tested immediately after production and after 50 days long storage at room temperature in desiccator. This study showed that 50 days long storage time did not influence negatively on quality of basil powders regarding all investigated parameters except moisture content which rose for 30 - 40 %.

Keywords: Ocimum basilicum, spray drying, dry powder characterization, storage time

Introduction

Sweet basil (*Ocimum basilicum* L.), a member of the *Lamiaceae* family, is native to Asia, Africa, South America, and the Mediterranean but widely cultivated commercially in many countries (Grayer et al., 1996) in natural and green house conditions in order to improve its yield and obtain a regular supply of the material (Johnson et al., 1999). Among 150 species of the genus *Ocimum*, basil is the major essential oil crop (Sajjadi, 2006) which has been extensively utilized in food as a flavoring agent (Telci et al., 2006), due to its foliage adding a distinctive flavor to many foods. Basil is also a rich source of aromatic compounds and essential oils containing biologically active compounds which possess insecticidal (Deshpande and Tipnis, 1997), nematicidal (Chatterjee et al., 1982), fungistatic (Reuveni et al., 1984) and antimicrobial properties (Wannissorn et al., 2005). According to its chemical composition, sweet basil belongs to aromatic herbs whose quality is determined by the content of essential oil. The essential oil content varies between 0.5 - 0.8 % (Tucakov, 1990). Essential oil comprises around 30 characteristic compounds, mostly terpenes (monoterpenes and sesquiterpenes, and their oxygenated derivates) and phenolic compounds. Dominant components of essential oil are primarily phenolic compounds: methylchavicol, linalool, eugenol, methyleugenol and methylcinnamate (Filip, 2014).

This aromatic herb has been used traditionally as a medicinal herb in the treatment of headaches, diarrhea, constipation, warts, worms and kidney malfunctions (Simon et al., 1999). The leaves and flowering tops of the plant are recognized as carminative, galactogogue, stomachic and antispasmodic in folk medicine (Sajjadi, 2006). It was reported previously that the leafy parts of basil had tonic and antiseptic activity (Kosekia et al., 2002). It is also known that leaves of basil are suitable for the treatment of pain and cough (Basilico and Basilico, 1999). In addition, basil is used for inflammations and dyspepsia (McClatchey, 1996). Recently, the potential uses of basil essential oil, particularly as antimicrobial and antioxidant agents have also been investigated (Lee et al., 2005, Politeo et al., 2007, Sartoratotto et al., 2004, Suppakul et al., 2003, Wannissorn et al., 2005). The basil essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Da-Silva et al., 2003, Sajjadi, 2006). Since sweet basil is scarce during off-seasons and highly perishable, it has to be preserved against deterioration and spoilage, which makes its drying a primary issue (Parmar et al., 2017). Spray drying is perceived as the most economic technique maintaining high quality of powder by rapid dehydration. It ensures a large surface area in the form of fine liquid droplets obtained through atomization in the drying chamber, which leads toward production of regularly and spherically shaped powder particles (Fazaeli et al., 2012; Turchiuli et al., 2011).

The main aim of this study was to assess the efficiency of spray drying to microencapsulate phenolic compounds from basil extracts obtained by maceration. In order to estimate influence of storage time on quality of spray dried extracts of basil, obtained powders were tested for moisture content, rehydration and bulk density immediately after their production and after 50 days long storage. In addition, the content of polyphenols in basil powders was determined promptly after their

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production and after 50 days of storage at room temperature.

Materials and methods

Plant material

Sweet basil (*O. basilicum* L.) was cultivated at the Department for Organic Production and Biodiversity, Bački Petrovac, Serbia. The aerial parts of basil were stored in a paper bags, at a room temperature. The dried basil was grounded in a domestic blender prior extraction, and the particle size of grounded material was determined using sieve sets (Erweka, Germany). Mean particle size of basil used in investigation was 0.2138 mm.

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin-Ciocalteu reagent and (±)-catechin were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Gallic acid was purchased from Sigma (St. Luis, MO, USA). All other chemicals and reagents were of analytical reagent grade.

Liquid extract and liquid feed preparations

Basil liquid extracts were generated by classical extraction technique-maceration with 50% ethanol. Plant material and 50% ethanol (ratio 1 g/10 mL) were mixed twice a day in a glass flask for 5 days. Extraction was performed at room temperature. After extraction, obtained extracts were immediately filtrated through filter paper under vacuum. Extracts were collected into glass flask and stored at 4 °C until further experiments. Maltodextrin (MD) of dextrose equivalent (DE) 16 was used as a carrier material and its solution was added to liquid feed in concentration of 10%, 20% and 30%, and both were mixed with a magnetic stirrer at temperature of approx. 40 °C prior to the spray drying (SD) process. Therefore, four basil powder samples were obtained (0% MD, 10% MD, 20% MD and 30% MD) and further investigated.

Spray drying process and its efficiency

The prepared liquid feed was spray dried using an Anhydro lab scale spray dryer (APV Anhydro AS, Denmark). A peristaltic pump was used to pump the feed into the dryer. Liquid feeds were dried at inlet temperature, T_i =120 °C, while outlet temperature, T_o was kept constant at 80 °C. During the production of the dry powder extract, atomizer's speed ranged from 20,000 to 21,000 rpm. The obtained powder was separated from air by a cyclone. Obtained dry extracts were collected in glass bottles, sealed and kept protected from air and humidity. The production yield of the SD process was determined according to the mass of total solids measured in the feed and the mass of dry powder obtained at the end of the process in powder receptacle.

Moisture content

Powder (dry extract) moisture content was carried out according to standard procedure described in European Pharmacopeia (Ph. Eur. 8). All experiments were performed in three replicates. Moisture content was determined in dry extracts of basil immediately after their production and after 50 days of storage in desiccator.

Rehydration

Rehydration of dry extracts was determined by adding 2 g of powder into 50 mL distilled water, at room temperature. Mixture of powder and water is mixed via magnetic stirrer in glass flask. Time needed for powder to completely rehydrates, expressed in seconds, represents rehydration time (Goula and Adamopoulos, 2005; Goula and Adamopoulos, 2008). Rehydration was determined in dry extracts of basil immediately after their production and after 50 days of storage in desiccator.

Bulk density

Bulk density was determined by measuring the volume of the powder mass. 20 g of basil powder was placed to a 100 mL graduated glass cylinder. The glass cylinder was held on the vibration plate for 2 min. After that, bulk density was calculated from the difference of the empty glass cylinder and the mass of the glass cylinder with powder (Vidović et al., 2014). Bulk density was expressed as mg of powder per mL. Bulk density was determined in basil powders immediately after their production and after 50 days of storage in desiccator.

Total phenols content

The contents of total phenolic compounds (TP) in herbal powders were determined by the Folin–Ciocalteu procedure (Kähkönen et al., 1999). TP was expressed as mg of gallic acid equivalent per g of dry extract (mg GAE/g DE). All experiments were performed in three replicates.

Total flavonoids content

The total flavonoids content (TF) was determined using aluminum chloride colorimetric assay (Markham, 1989), using catechin as a standard compound. The content of total flavonoids was expressed as mg of catechin equivalent per g of dry extract (mg CE/g DE). All experiments were performed in three replicates.

Results and discussion

Process efficiency

Several drying techniques (spray drying, vacuum drying, freeze drying and spouted bed drying) have been proposed for the production of dry extracts on the industrial scale. According to Hammami and Rene (1997), an industrial scale comparison showed that the spray drying process is around 4-5 fold more economic than that of freeze drying due to its less electricity consumption and short drying time. Santivarangkna et al. (2007) reported that spray drying is 8-fold more economic than freeze drying and 4-fold more economic than vacuum drying. Since inlet temperature of the drying air affects the process of the liquid removal from the dispersion, it is necessary to adjust this temperature to allow the best possible thermal efficiency of the process, without the risk of destroying the product. The drying air temperature and air humidity simultaneously affect the final solvent content in the product, however the temperature is the only variable that can be changed at any time. Also, the humidity of the inlet air has a significant effect on the performance and efficacy of the drying process (Baker, 1997).

In our study 50% ethanolic extract, obtained by maceration of basil, was used as liquid feed. One powder was obtained with no added maltodextrin (0% MD), while other three powders were prepared with addition of 10%, 20% and 30% maltodextrin (10% MD, 20% MD and 30 % MD). Maltodextrin of dextrose equivalent (DE) 16 was used as a carrier material. As stated in the literature, maltodextrins with a DE in the range from 10 to 20 proved to be the best for carriers in spray dried powders due to less turbidity at high concentrations (Raja et al., 1989). Peng et al. (2013) observed that maltodextrin was superior to β-cyclodextrin for protecting the antioxidant components. The carrier material also needs to be inexpensive, food grade, readily available, and legally allowed (Mahdavi et al., 2014). Partanen et al. (2002) observed that maltodextrin was more heat stable than β–cyclodextrin under dry conditions. According to Bhandari et al. (1997), recovery higher than 50% in the cyclone is regarded as the criteria of efficient drying in lab-scale dryers.

The efficiency of four investigated spray drying processes can be considered high since in all cases it was above 50% (0% MD: 53.80%; 10% MD: 64.15%; 20% MD: 67.18%; 30% MD: 69.66%) (Table 1). In addition, efficiency of microencapsulation was increased by adding maltodextrin which can be related to the effect of carrier's concentration on the formation of surface core prior to the formation of crust around the drying droplets (Young et al., 1993).

Table 1. Process efficiency for basil powder samples

Basil powder	Process efficiency [%]
0% MD	53.80
10% MD	64.15
20% MD	67.18
30% MD	69.66

Basil powder properties

Moisture content

Moisture content is essential factor which affects stability, particle size, morphology and rheological properties of powders (Bhandari and Hartel, 2005). The spray dried product is highly stable, due to its low moisture content and water activity. Common ranges of moisture content and water activity of spray dried fruit and vegetable powders are 2 – 5 % and 0.2 - 0.6, respectively (Shishir et al., 2016; Patil et al., 2014; Tze et al., 2012). Under these conditions, the powdered products are rather resistant to microbiological and oxidative degradation, i.e. browning and hydrolitical reactions, lipid oxidation, auto-oxidation and other enzymatic activities (Marques et al., 2007; Tan et al., 2011b). Moisture contents of basil powders are presented in Table 2.

Table 2. Moisture content in basil powders determined immediately after production and after 50 days of storage

Basil powder	Moisture content [%]	Moisture content after 50 days [%]
0% MD	7.97	11.66
10% MD	5.96	10.57
20% MD	7.83	11.11
30% MD	8.48	10.20

Moisture content of obtained basil powders ranged between 5.96 and 8.48 %, which is similar to moisture content of *A. millefollium* powders (6.10 - 7.68 %) (Vladic et al., 2016). The lowest moisture content was determined in powder with 10% MD, whereas the highest maltodextrin concentration provided powders with highest moisture content. However, after 50 days of storage, moisture content deteriorated and rose for 30 - 40 % except in the case of 30% MD sample where it increased for 16.86%. It can be concluded that the highest carrier concentration exhibited the highest efficacy with regard to content of moisture in powders during storage time.

Rehydration time

Rehydration is a significant step in the utilization of dried fruits and vegetables. Since consumers have shown an increased interest in healthy and ready-to-use foods (De Belie, Laustsen et al., 2002), convenience, freshness, high quality, flavor (Hollingsworth, 2002) and adequate reconstitution are essential in meeting their expectations. Optimal reconstitution conditions are of outmost importance since pre-drying treatments, drying and rehydration processes induce many changes in the structure and composition of plant tissue which result in impaired reconstitution properties (Lewicki, 1998). Optimal reconstitution can be achieved by controlling the drying process and adjustment of the rehydration conditions (Marabi et al., 2004, Marabi et al., 2003, Marabi and Saguy, 2004). Rehydration time in basil powders is presented in Table 3.

Table 3. Rehydration time in basil powders determined immediately after production and after 50 days of storage

Basil powder	**Rehydratation time** [s]	**Rehydratation time after 50 days** [s]
0% MD	6	11.2
10% MD	8	12.5
20% MD	8.2	13.2
30% MD	9.1	18.3

Powders are intended for rehydration with water or, respectively, an aqueous liquid. An ideal powder should be wetted quickly and thoroughly, sink into the liquid rather than float on the surface and disperse/dissolve within a short period of time without lump formation. This ideal behaviour is difficult to achieve, since the manufacturing processes usually yield particles of rather small size and/or unfavourable structure (Hogekamp and Schubert, 2003). In our study, rehydration tends to rise with addition of carrier. Rehydration ranged between 6-9.1 s, which is rather satisfying and deteriorated after 50 days of storage from 11.2 to 18.3 s.

Bulk density

Bulk density is a very important parameter to characterize powders which have to meet bulk density targets to provide consistent weight during packaging (Legako & Dunford, 2010). Measurement of bulk density of herbal extracts is particularly significant due to its further use in the formulation of final pharmaceutical product which is restricted in volume (Goula and Adamopulos, 2010). The preferable powder properties for packaging and storage are higher bulk density and low moisture content (Shishir and Chen, 2017). Tze et al. (2012) reported that the bulk density is associated with the particle size. Smaller particles reduced the void spaces among them and arranged the particles in a close form, therefore the lower particle size led to higher bulk density (Tze et al., 2012). The bulk densities in investigated basil powders were 48 mg/mL in carrier-free powder, 40 mg/mL in both 10% MD and 20% MD powders and 51 mg/mL in 30% MD powder (Table 4). These values are lower than the one obtained in *S. montana* powder by adding 10% MD (82.4 mg/mL) (Vidovic et al., 2014), but in agreement with bulk density measured in *A. millefollium* powder with 10% MD addition (41.31 mg/mL) (Vladic et al., 2016). After 50 days of storage, bulk density of powders increased. The most significant rise was determined in the powder with 30% MD (app. 2-fold), while in the case of powder with 20% MD bulk density increased slightly.

Table 4. Bulk density in basil powders determined immediately after production and after 50 days of storage

Basil powder	Bulk density [mg/ml]	Bulk density after 50 days [mg/ml]
0% MD	48	65
10% MD	40	51
20% MD	40	42
30% MD	51	101

Polyphenol content in basil powders

Polyphenols are abundant micronutrients in our diet, and proof for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The health effects of polyphenols depend on the amount consumed and on their bioavailability. Knowledge of the bioavailability and metabolism of the various polyphenols is necessary to evaluate their biological activity within target tissues (Manach et al., 2004). A large pool of preclinical research and epidemiological data suggests that plant polyphenols can slow the progression of certain cancers, reduce the risks of cardiovascular disease, neurodegenerative diseases, diabetes, or osteoporosis, suggesting that plant polyphenols might act as potential chemopreventive and anti-cancer agents in humans (Arts and Hollman, 2005; Scalbert et al., 2005; Scalbert et al., 2005; Surh, 2003). Since high bioactive potential has been assigned to polyphenolic compounds, it is necessary to determine their content in basil dry extracts which could be further implemented in pharmaceutical formulations and dietary supplements. Polyphenol contents in obtained basil powders are presented in Table 5.

Table 5. Total phenols (TP) and total flavonoids (TF) contents of basil powders determined immediately after production and after 50 days of storage

Basil powder	TP [mg GAE/g DE]	TP after 50 days [mg GAE/g DE]	TF [mg CE/g DE]	TF after 50 days [mg CE/g DE]
0% MD	148.55	139.59	109.83	93.03
10% MD	135.32	127.68	106.48	90.60
20% MD	133.45	125.36	84.98	78.36
30% MD	113.63	111.21	81.24	80.04

The highest value of total phenols in basil powders (148.55 mg GAE/g DE) was obtained in carrier-free powder, while the lowest value of TP was gained in 30% MD sample. This outcome is expected since sample is diluted with addition of carrier. TP value obtained in 0% MD is comparable with TP values obtained in *S. montana* powder with 10% MD (153.61 mg GAE/g) and *A. millefolium* powder with 10% MD (151.86 mg GAE/g). After 50 days of storage at room temperature in desiccator TP in all samples deteriorated and decreased for 2 – 6 %.

Total content of flavonoids was in the range from 111.21 to 139.59 mg GAE/g. As in the case of TP, TF content also decreased with the addition of maltodextrin due to dilution of dry extracts' bioactive compounds with carrier. Determined values were lower than the one obtained in *S. montana* powder with 10% MD (118.69 mg CE/g). Total flavonoids in spray dried rosemary extracts obtained by maceration with aqueous ethanol ranged between 46 and 76.4 mg/g (Couto et al., 2012). Pavlic et al. (2017) investigated polyphenolic contents in two powders (carrier-free and powder with 20% MD) of *Salvia officinalis* obtained by spray drying of subcritical water extracts. They reported lower values for total phenols (106.26 mg GAE/g for carrier-free powder and 91.35 mg GAE/g for 20% MD added powder) and total flavonoids (58.97 mg CE/g for carrier free powder and 56.98 mg g CE/g for 20% MD added powder). After 50 days of storage at room temperature in desiccator TF in all samples deteriorated and decreased by 1.5 – 15 % and 30% was the most efficient concentration of carrier for preservation of polyphenols.

Conclusions

Spray drying is a well-established technique for obtaining powders from fruit juices but not so widespread when liquid feed is prepared as water/hydroalcoholic extract of herbal material. The major challenge in spray drying is to produce a standardized herbal dried extract that has the desired content of bioavailable active compounds. This is

particularly difficult since herbal extracts contain number of chemical constituents and are inconsistent in composition. This study has shown that 50 days long storage time did not influence negatively on quality of basil powders regarding all investigated parameters except moisture content. After 50 days of storage, moisture content significantly deteriorated and rose for 30 – 40 %. On the contrary, TP and TF contents in all samples slightly deteriorated and decreased for less than 15% after 50 days of storage at ambient temperature.

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