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Effect of Drying Processes on Physical and Chemical Properties and Antioxidant Capacity of Squid *(Sepia pharaonis)* Ink Powder

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ABSTRACT: Squid is a popular fishery product for local consumption in Thailand and export. The ink sacs are by-products of processing and can be a source of bioactive compounds. Drying is widely used in food processing and preservation. But drying process may cause irreversible changes of bioactive compounds. Three methods of drying were tested, including foam mat drying, freeze drying and spray drying, on antioxidant activities, moisture content, water activity, lightness, rehydration ratio, water absorption index and water solute ability of squid ink powders. The different drying methods had impacted on physical and chemical properties significantly ($p \leq 0.05$). Foam mat drying had the greatest effect on water solute ability at 17.44% compared to 5.53 for freeze drying and 8.98 for spray drying. The antioxidant capacity of squid ink powder was determined by DPPH, ABTS radical scavenging activities and metal chelating activity. The highest antioxidant activity was foam mat drying method had DPPH, ABTS radical scavenging and metal chelating activities of 668.96± 72.37, 3072.8±68.07 μmol TE/g sample and 468.63±32.54 μmol EE/g sample, respectively.

Keyword: squid ink, drying, antioxidant activity

INTRODUCTION

Squid and cuttlefish are the important fishery products of Thailand, as well as other Southeast Asian countries, such as Japan [1]and are exported worldwide.

Squid are coleoid cephalopods, which produce ink, which is colored by melanin, but it also contains other constituents. It has been used by humans in various ways from time immemorial [2]. Sepia ink is largely contains of water but it also contains about 5–8% protein and about 15% of melanin [3,4], which gives it the distinctive color. Other constituents that have been reported include: L-dopa, dopamine and taurine, but may vary depending on the extraction method [2]. During processing most squid ink is discarded. The ink sac was generated as by-products in local market and can create environmental pollution without appropriate to handle. These by-products can be a source of bioactive compounds [11]. Squid ink is widely used in Italian and Spanish cuisine, and is also now being used more frequently in Southeast Asian cooking. Squid ink can be added as a sauce to several dishes, such as risottos, where it adds an inky black sheen to foods and a deep flavor. Other preparations include adding the ink into the starch, such as with squid ink pasta, making for black pasta that shows off the bright colors of vegetables. Powder products have many benefits more than liquid counterparts such as reduced volume or weight, reduced packaging, easier handling, transportation and longer shelf life [5,6].

However, drying process may cause irreversible changes of bioactive compounds.
Squid ink may also have medicinal properties. For example, the methanol extract of squid ink showed exhibited significant activity in the control of the tuberculosis organism [7].

The present study was to investigate the effect of drying method using foam mat-, freeze- and spray drying on physico-chemical and antioxidant properties of squid ink.

MATERIAL AND METHODS

Preparation of sample

Squid ink sacs were obtained from a local market in Bangkok, Thailand and transported to the faculty of Agro-Industry, KMITL packed in ice that contained with ice: squid ratio of 2:1 (w/w). The squid ink was extracted by cutting the ink sac and then squeezing out the ink.

Drying process

Foam mat drying processing

1% of hydroxypropyl methyl cellulose (HPMC), 1% of carboxymethyl cellulose(CMC) and 25% of maltodextrin in ratio 3 : 2 : 2 : 1 by weight was added to the squid ink and blended using a blender (Philips hand mixer, Thailand) at the highest speed for 10 minutes. It was then dried at 60°C for 270 minutes in a tray drier and milled to a sieve size of 0.25 mm (Pin mill, ZM 200, Thailand) and stored in a desiccator.

Freeze drying processing

Squid ink was plastered on a tray with a depth of 50 mm and placed in an air blast freezer (BCF-50-RE, Thailand) at -40 °C for 180 minutes. Thereafter, it was dried in a freeze dryer (CoolSafe, Thailand) for 40 hours.

Spray drying

Squid ink was diluted to 30^{O}brix with distilled water and then filtered through a 60 mesh sieve and dried in a spray dryer (SDE-5, Euro best technology, Thailand). Inlet and outlet temperature was set at 130°C and, 100°C, respectively, with a feed rate of 5 rpm. The powder was then kept in a desiccator until analyzed.

Extraction of squid powders

The squid ink and squid powder from each drying method were diluted ten fold using deionized water. Sample solutions were extracted using a sonicate bath (ELMA, S30H, Germany) at a frequency of 37 kHz and a temperature below 5°C for 15 minutes and then centrifuged at 11,000 rpm for 20 minutes at 4°C using a refrigerated centrifuge (Centrifuge, Eppendorf, 5804 R, Germany) [8]. The supernatant was then analyse as shown below.

Determination of Antioxidant activities
DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extracts was determined using the method described by [9-11] with slight modifications. Briefly, 1.5 mL of extract was mixed with 1.5 mL absolute ethanol solution containing 0.15 mM DPPH. The mixture was shaken vigorously and then left to stand for 30 min in the dark. The absorbance was measured at 517 nm using double beam spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The blank was prepared in the same manner, except that deionized water was used instead of the sample. A standard curve was prepared using Trolox in the range of 0 to 50 μM. The activity was expressed as micromole Trolox equivalents (TE)/g sample.

ABTS radical scavenging activity

The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity of extract was determined by the method of [9-11] with slight modifications. ABTS was dissolved in water to a 7.4 mM concentration and. ABTS radical cations were produced by reacting ABTS stock solution with 2.6 mM potassium persulfate and mixing the two stock solutions in equal quantities and allowed to stand in the dark at room temperature (±25°C)for 14 hours before use. The solution was diluted by mixing 2.5 mL of ABTS solution with 100 mL of absolute ethanol in order to obtain an absorbance 0.70±0.02 at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared daily. A sample (150 µL) was mixed with 2,850 µL of ABTS solution and the mixture was left at room temperature for 10 min in the dark. The absorbance was then measured at 734 nm. A standard curve of Trolox ranging from 0 to 1000 µM was prepared. The activity was expressed as micromole Trolox equivalents (TE)/g sample.

Chelating activity

The chelating activity toward Fe^{2+} extract was determined by the method described by [12]. The sample (4.7 mL) was mixed with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature and the absorbance was then read at 562 nm. The control was prepared in the same manner except that deionized water was used instead of the sample. A standard curve (0 to 50 µM EDTA) was prepared. The Fe^{2+} chelating activity was expressed as EDTA equivalents (µmol EDTA equivalents (EE)/g sample).

Chemical and Physical analysis

Water solubility index (WSI) and water absorption index (WAI)

The WSI of the powder was determined using the modify method described by [13]. Squid ink powder (2.5 g) and distilled water (30 mL) were vigorously mixed in a 250 mL flask, stirrer 200 rpm for 30 min and then centrifuged for 10 min at 6000 rpm (Centrifuge, Eppendorf, 5804 R, Germany). The supernatant was carefully collected in a pre-weighed beaker and oven dried at 105±2°C until the weight was stable. The WSI (%) was calculated as the percentage of dried supernatant with respect to the amount of the original squid ink powder. The WAI (g/g) was calculated as the ratio of the amount of precipitate and the original weight of squid ink powder.

Moisture content and Water activity (aw)

Determination of moisture content was according to AOAC (2000). The sample (2 g) was placed in an aluminum can and dried in an oven (UM 500, MEMMERT, Germany) at 103 ± 2°C until the weight was stable. The moisture content was calculated in term of percentage.

The water activity (a_w) was determined in triplicate, using a water activity meter (Aqualab, Decagon Devices, USA) at 25°C.

Rehydration ratio

Rehydration ratio was determined by the method described by [14] with slight modifications. The squid powder 2.5 g was diluted with distilled water (100 ml). The solution was boiled using a hot plate stirrer (Cimarec, USA) at 100°C for 10 min and then centrifuged for 10 min at 6000 rpm. The rehydration ratio was calculated as the ratio of the amount of the precipitate and the original weight of squid ink powder.

Color

The color of the samples was measured using a Chroma Meter a (Minolta, Model CR-400, Japan) and represented as the average in terms of lightness (L^*), where 0 = black and 100= white.

Statistical analysis

The means and standard deviations of the results were calculated from three replicates. One-way ANOVA (analysis of variance) at the level of significance $p \leq 0.05$ using SPSS Version 24.0 was performed for comparison of the means.

RESULT AND DISCUSSION

Chemical and physical properties

The results of different drying methods on physical and chemical properties of squid ink powders are shown in Table 1**.**

The moisture content and water activity of freeze dried squid ink powder was significantly ($p \leq 0.05$) higher than those that had been spray dried and foam mat dried (Table 1). This is because of the resistance to water vapour flow during sublimation. This parameter is closely related to the composition, concentration and percentage of solids in the material subjected to drying. The more solids in the material, the greater the barrier to mass transfer during freeze drying which has a direct impact on the sublimation rate [15,16].

Water activities of squid ink powders varied significantly, ranging from 0.24 to 0.31. In terms of microbial stability, water activities of all treatments were sufficiently low to ensure safe storage stability as limit for yeast is below than 0.88, for spoilage molds is below than 0.70 and the absolute limit for all organisms is below than 0.60 [17].

Table 1 Effect drying methods on chemical and physical properties of squid ink powders.

Drying method	Foam mat drying	Freeze drying	Spray drying
Moisture content (%)	10.23 ± 0.08^{a}	11.29 ± 0.50^{b}	11.07 ± 0.54^{ab}
Water activity (a_W)	0.31 ± 0.003^{a}	0.29 ± 0.003^{b}	0.24 ± 0.005^{c}
Water solubility index	17.44 ± 0.12^{a}	5.53 ± 0.10^{b}	8.98 ± 0.08^{c}
Water absorption index	2.39 ± 0.04^{a}	2.55 ± 0.02^{b}	2.49 ± 0.02^{c}
Rehydration ratio	2.61 ± 0.08^{a}	2.66 ± 0.17^{a}	2.55 ± 0.05^{a}
Lightness (L*)	28.8933 ± 0.28^{a}	28.0067 ± 0.31^{b}	28.4433 ± 0.43^{ab}

Mean±SD (n=3). Different letter within the same row were significantly different ($p \leq 0.05$).

The water solubility index measures the amount of soluble components released from the sample. The drying of squid ink powders affected solubility index and water absorption significantly ($p \leq 0.05$). Water absorption index was by far the highest for those squid ink powders from foam mat drying followed by spray drying and freeze drying (Table 1). The previous results who studied feasibility of *Nigella sativa* powder by foam mat drying, reported that at higher temperatures can increase the porosity of the sample and higher of porosity leads to a larger surface area of contact between the powder and water [18,19].

The water absorption index is a measure of the powders' ability to reconstitute to a liquid. Squid ink powders from foam mat drying had water absorption lower than

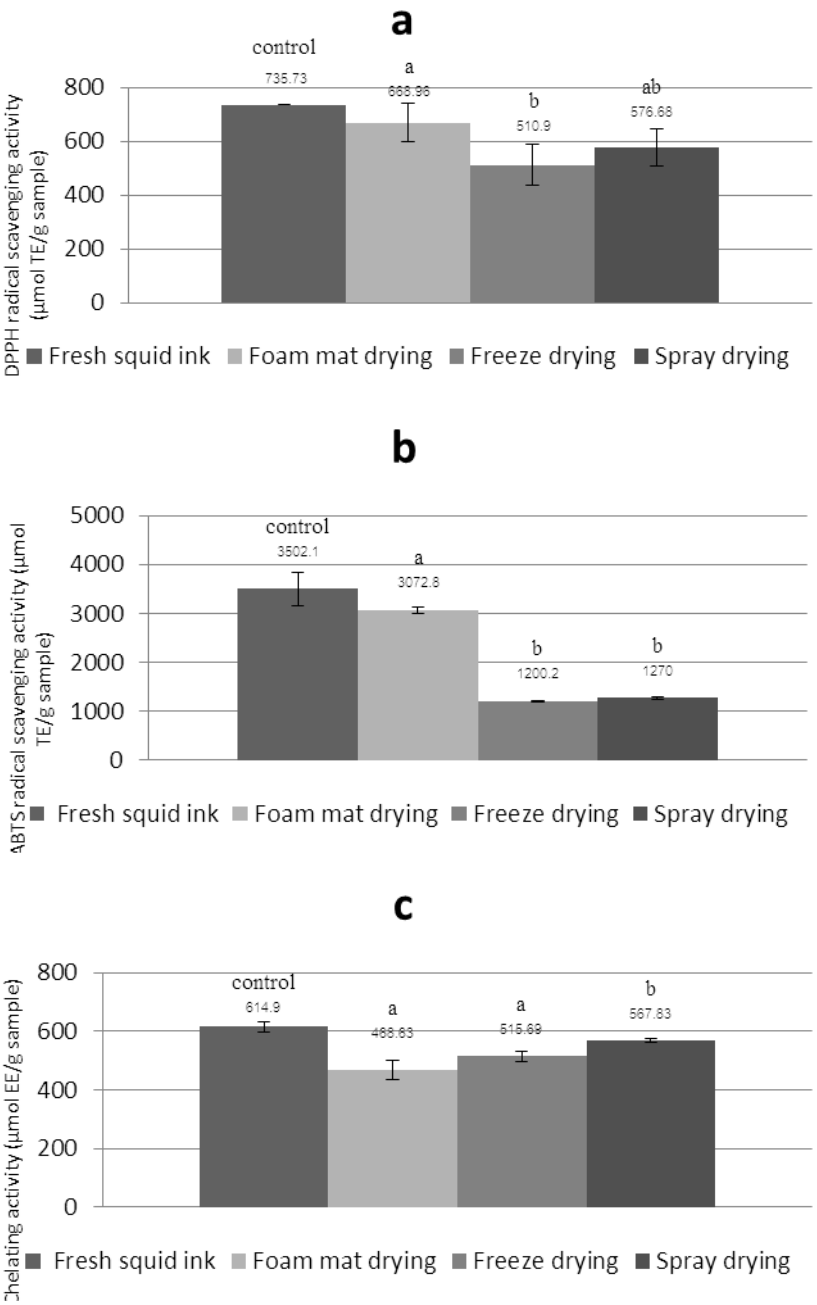

Figure 1 DPPH, ABTS radical scavenging activity and chelating activity of different drying method of squid ink powders. DPPH (a), ABTS (b), chelating activity (c). Bars represent the standard deviation (n=3). Different letters on the bars for each assay indicate significant differences ($p \leq 0.05$).

spray drying and freeze drying (Table 1). The previous study who compared various drying temperature and foam thicknesses found that increase the drying temperature and foam thickness also decreases the water absorption index [19].

The rehydration ratios of the three drying methods tested were not significantly different ($p \leq 0.05$) (Table 1). In previous study, showed that freeze drying resulted in the highest rehydration ratio of the drying methods they tested, and also lower drying

temperatures tend to increase rehydration capacity [20].

Squid ink powders from foam mat drying were lighter than lightness from spray drying and freeze drying (Table 1). Due to Addition of maltodextrin increase in the lightness of the powders, which is most likely a reflection of the dilution of the original sample with a white powder of maltodextrin [21]. However, inspite of the *L** values showing some significant differences these were only small and would not affect the acceptability of the dried powder.

Antioxidant activities of squid ink powders

The higher antioxidant activity was from foam mat drying ($p \leq 0.05$). The foam mat drying had higher activity than spray drying and freeze drying. ABTS radical scavenging activity (Figure 1b) was similar to DPPH radical scavenging activity (Figure 1a) but ABTS radical scavenging activity of foam mat drying showed double the activity than spray drying and freeze drying ($p \leq 0.05$). Squid ink was previously reported to contain L-dopa and dopamine at concentrations of 1.15 and 0.19 mM, respectively [22]. The hydroxyl groups of those compounds more likely donated hydrogen atoms to the tested radicals. In previous study who compared air-drying temperature on extractable of lime wastes reported that DPPH and ABTS capacity showed higher activities when temperatures were increased [23]. Metal chelating activity of different drying methods (Figure 1c) showed that the activity of foam mat drying and freeze drying was not significantly different ($p \leq 0.05$). The higher activity was from spray drying. The activity of foam mat drying and freeze drying not significantly different ($p \leq 0.05$). This result indicates that squid ink acted as a reducing agent, which provided electrons for stabilization or could chelate prooxidative metals[11]

CONCLUSIONS

This study was carried out to determine the effect different methods of drying squid ink powders on their antioxidant activities and physical and chemical properties. The highest levels of physical and chemical properties, including antioxidant activity, were from foam mat drying. Therefore it is concluded that, of the three methods tested, foam mat drying gave the best results and could provide a useful by-product for the squid processing industry.

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