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Title: Flavor Retention Characteristics of Amorphous Solid Dispersion of Flavors, Prepared by ~~Different~~ Vacuum-Foam- and Spray-Drying ~~Methods and~~ under Different Conditions

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Running Title: AMORPHOUS SOLID DISPERSION OF FLAVORS

ABSTRACT

We investigated the powderization of flavoring substances, using an amorphous solid dispersion (ASD) technique, in which hydrophobic molecules are separately embedded in a water-soluble carrier matrix. Six flavors, five carrier forming materials (polyvinylpyrrolidone/ disaccharides), two solvents (methanol/ ethanol) and two drying methods (vacuum-foam-/ spray-drying) were employed. The drying conditions for the two drying processes were first examined, and under the optimal drying conditions, various flavor-carrier combinations and compositions of ASD samples were prepared and their flavor retention after drying and during storage under a vacuum were compared. Results demonstrated that flavor loss during drying and storage was minimized when the material was vacuum-foam-dried with polyvinylpyrrolidone. Vacuum-foam-drying in the presence of α-maltose or palatinose also resulted in a greater retention of flavor during drying and storage than a typical O/W emulsification-based powderization. These findings suggest that the ASD-based powderization of flavoring materials is a feasible alternative to the currently used produces.

Key words: flavor, amorphous solid dispersion, vacuum foam drying, spray drying, polyvinylpyrrolidone, disaccharide

1. Introduction

Flavoring substances, ~~which are commercially~~ commonly used in the food manufacturing industries, often are in a liquid oil~~s~~ form and ~~it is necessary~~ need to be converted ~~them~~ into a powdered form for easier ~~of~~ handling. The powderization of a flavoring oil is often achieved through ~~the use of~~ an O/W emulsification process using ~~with~~ surface active ingredients, followed by ~~and subsequent~~ drying in the presence of a wall material such as a carbohydrate and a protein.[1-4] Many reviews have been published on the emulsification-based-powderization of oily materials~~ have appeared~~,[2,4,5,6] and ~~further attempts~~ additional efforts to improve the oil-powderization process have been reported.[7-9]

The implementation of the emulsification-based-powderization of flavor oils is inevitably accompanied by intensive investigations of the product formulation and the conditions used for emulsification and drying.[10,11] One possible strategy to reduce the trial-and-error labor required to obtain a commercially viable flavor powder product is to simplify the product formulation. Namely, when the powdered flavor product can be made only by a flavor and a carrier excipient component, the parameters associated with the product composition would be limited only to the concentrations of the flavor and carrier components; The points to be examined in the case of the drying process would be also reduced to only two components of concern.

One attempt to simplify the development of the flavor powder products involved the use of cyclodextrins.[12,13] In this procedure, the flavor molecule is included in the hydrophobic cavity of the cyclodextrin molecule, which allows the use of a surface-active substance to be omitted. As an alternative approach to this issue, we ~~employed~~ utilized the amorphous solid dispersion (ASD) technique,[14] ~~that~~ which is frequently used to fabricate mixtures of a hydrophobic drug and a carrier excipient at the molecular level in the pharmaceutical field.[15-17] In the case of ASD-based flavor powderization,[14] a disaccharide such as α-maltose or trehalose was amorphized by being dried from an aqueous solution. ~~and~~ It was then dissolved in methanol together with the flavoring substance, followed by drying under reduced pressure.[18-20] The results demonstrated that a flavor substance (cinnamaldehyde) could be stably embedded in the methanol-dried amorphous sugar matrix.[14] The yield ~~in~~ of this flavor powderization, namely, the amount of flavoring oil encapsulated in the resulting ASD powder, was higher ~~greater~~ than that for powderization via O/W emulsification when using the appropriate types of sugar (α-maltose and trehalose) ~~were used~~ as a carrier matrix.[14] Another unique aspect of ASD-based flavor powderization was that the flavor molecules were dispersed one by one throughout the matrix.[14] This provides a novel function in which the flavor molecules are simultaneously released at the moment that the ASD preparation is dissolved in an aqueous system.

As the next step, in this study, we focused on the impact of the powderization (drying) conditions and types of carrier excipient on the yield of flavor encapsulation in the ASD preparation. Six types of flavoring substances were used, and two drying techniques, namely, vacuum-foam-drying and spray-drying~~s~~, were employed in this study. As a carrier forming material, polyvinylpyrrolidone (PVP), which is typically used for preparation of the ASD of hydrophobic drugs,[15,21,22] and five types of disaccharides were used. We first investigated the drying conditions for the two drying methods. In the second step of this study, under the optimized drying conditions that were determined, flavor ASD experiments were conducted to compare the flavor encapsulation yields among the different ASD conditions, including the drying method, the type of carrier forming material and the solvent type. Based on the ~~obtained~~ findings obtained, the feasibility of using the ASD-based technique~~,~~ as an alternative to powderizing flavoring substances~~,~~ is discussed.

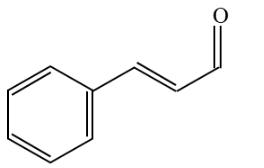
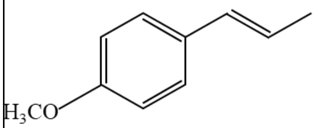
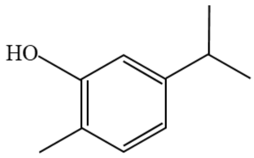
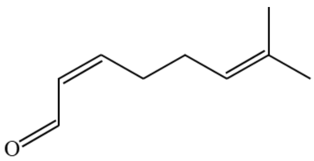
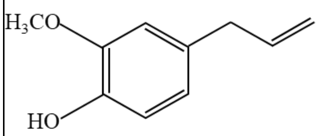
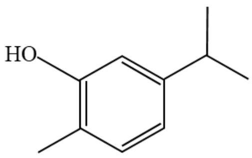
2. Materials and methods

2.1. Materials

Four types of disaccharides, α-maltose, palatinose, sucrose, and trehalose, which were products of FUJIFILM Wako Pure Chemical Co., (Osaka, Japan). These disaccharides were preliminarily amorphized before use by being freeze-dried and then thoroughly dehydrated following the same procedures as was used in our previous study.[23] Polyvinylpyrrolidone (PVP) having MW of ~24,500 Da was purchased from Nacalai Tesque (Kyoto, Japan). Cinnamaldehyde, anethole, citral (FUJIFILM Wako Pure Chemical Co.), eugenol (Sigma-Aldrich Co., St. Lous, MO) carvacrol, rasberry ketone

(Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were used as flavoring components (Table 1). Methanol and ethanol were obtained from Nacalai Tesque (Kyoto, Japan). Ryoto sugar ester L-1695 (Mitsubishi Chemical Foods Co., Tokyo, Japan), composed of 80% sucrose mono- and 20% di- + tri-laurate, was used to emulsify and then powderize (spray-dry) the aqueous suspension of the flavor oil. All of these chemicals were reagent grade and were used without further purification.

Table 1. Flavoring substances used in this study. Their boiling points and vapor pressure are also listed. Wavelength values in square brackets represent those used to measure the absorbance of the flavoring substance in methanol.

Flavor (Cat. No.)	Structure	M.W.	Boiling point (˚C)	Vapor pressure (Pa)
cinnamaldehyde (031-03453[a])		132.2 [285 nm]	248[d], 253[e]	3.85[k]
anethole (010-03982[a])		148.2 [258 nm]	234[f]	6.66[l]
carvacrol (C0026[b])		150.2 [275 nm]	237-238[g]	6.4[m]
citral (032-05982[a])		152.2 [238 nm]	229[h]	12.2[n]
eugenol (E51791[c])		164.2 [230 nm]	256[i]	2.95[p], 3.9[m]
raspberry ketone (H0604[b])		164.2 [278 nm]	292[j]	0.0018[q]

[a] FUJIFILM Wako Pure Chemical Co.; [b] Tokyo Chemical Industry Co., Ltd.; [c] Sigma-Aldrich Co.; [d] Munde et al., 2021; [e] Lide, 1990; [f] Lide, 1994; [g] Merck Index, 1976; [h] Lide, 2005; [i] Widayat et al., 2014; [j] Raspberry ketone MSDS (Chinese); [k] Perry & Green, 1984; [l] https://www.takasago.com/en/node/663; [m] Ben Arfa et al., 2006; [n] van Roon et al., 2005; [p] https://haz-map.com/Agents/3697; [q] Park et al., 2016.

2.3. Preparation of Amorphous Solid Dispersions of Flavors

A 10 mL portion of methanol (or alternatively ethanol), containing 10~30 wt% of the flavoring substance, was added to a glass vial with carrier matrix-forming substance, namely, a disaccharide (as mentioned above) or PVP, to give a final concentration of 100 mg/mL. The mixed solution of flavor and carrier forming material was vigorously stirred with a vortex mixer for 2~3 minutes, and immediately thereafter, dried by either of the two drying methods as described below.

(1) Vacuum-foam-drying: Twelve 100 μL aliquots of the prepared flavor/carrier mixed solution were transferred to polypropylene 1.5 mL tubes and subjected to an "Initial drying" under reduced pressure (~2,000 Pa) with a centrifuge (3250 rpm, 473g) for 0~120 min. In the initial and secondary drying steps, a EYELA CVE-1010 centrifugal concentrator (TOKYO RIKAKIKAI Co., Tokyo, Japan) connected to an ULVAC FDU-1200 diaphragm type vacuum pump (ULVAC Japan, Ltd., Tokyo, Japan) were used, and the temperature of the drying chamber was set to 30±1˚C. After terminating the initial drying, the remaining sample solutions were punctured with a stainless steel needle (needle stimulation),[18,19] immediately followed by being dried again under the reduced pressure (ca. 2,000 Pa) (Secondary drying). The solution did not foam during the initial drying, and the stimulation of the remaining solution with the needle followed by the restart of vacuum drying (secondary drying) induced the solution to foam, depending on the initial drying conditions. The probability of inducing foaming by needle stimulation under different conditions were determined as the ratio of the number of the foamed aliquots to the total.[18] In the secondary drying, the same centrifugal concentrating set up as was used for the initial drying was used, but centrifugation was not applied because centrifugation often serves to inhibit solution foaming.[19]

(2) Spray-drying: The spray drying of the flavor/carrier solutions were conducted using a Yamato ADL-311S spray-dryer (Yamato Scientific Co., Ltd., Tokyo, Japan). Namely,

the prepared flavor/carrier solution was introduced into the spray-dryer at the rate of 1.6~7.5 mL/min and sprayed from a two-fluid nozzle atomizer at an atomization gas pressure of 0.1 MPa, followed by being dried by 0.68 m^3/min downstream hot air. The inlet temperature for the hot air was varied from 80~180˚C and then fixed at 160˚C, in which the outlet temperature was 85±5˚C. The spray-dried powders were separated from the air by a glass cyclone and collected in a glass collection container.

2.4. Flavor Oil Powderization via Drying of O/W Emulsion

Alternatively, the flavor (cinnamaldehyde) oil, surfactant (Ryoto Sucrose ester L-1695), and α-maltose were added to water at final concentrations of 10 mg/mL, 1 mg/mL, and 100 mg/mL, respectively. The mixed solution was converted into an O/W-type emulsion by homogenization at 10,000 rpm for 2 min using a Nissei Excel Auto-homogenizer DX-4 (Nihonseiki Co., Tokyo, Japan). The resulting flavor oil-in-water emulsion was spray-dried at an inlet air temperature of 120˚C in the same manner as for the ASD samples (2.3. (2)).

2.5. Storage of Amorphous Solid Dispersions of Flavors

The prepared ASD samples containing flavoring substances were stored over silica gel at 30±2˚C in a vacuum desiccator (~1 kPa). After appropriate storage, the amounts of flavoring substance remaining in the samples were quantified as described below and the storage stability of encapsulated flavor among the samples prepared under different conditions was compared.

2.6. Estimation of Flavor Retention in Amorphous Solid Dispersions

The flavoring component retained in the ASDs of flavors and in the dried O/W emulsion samples were quantified immediately after drying and after storage over silica gel for 1~7 days in the same manner as was used in our previous study.[14] In a typical run, the sample to be analyzed was suspended in known volumes (1-5 mL) of methanol to thoroughly extract the flavoring component, and the UV absorbance due to the flavor in the methanol was then measured, followed by being converted into the remaining amount of the flavor in the sample. The wavelengths for the measurements were 285 nm (cinnamaldehyde), 260 nm (anethole), 240 nm (citral), 235 nm (eugenol), 225 nm (rasberry ketone), and 277.5 nm (carvacrol), respectively.

2.7. Scanning Electron Microscopy

The vacuum foam or spray dried amorphous solid dispersions of flavor were observed by scanning electron microscopy (SEM) as was described in our previous study.[30] Namely, the ASD powder was fixed on the SEM sample stage with carbon double-sided tape and then coated with a thin film (ca. 40 nm) by evaporating a Pt/Pd alloy using a Hitachi E-1030 ion sputter instrument (Hitachi High-Technologies Co., Tokyo, Japan). The SEM images of the ASD powders were collected using a KEYENCE VE9800 scanning electron microscope system (KEYENCE Co., Tokyo, Japan) at an accelerating voltage of 15 kV.

2.8. *Differential scanning calorimetry*

A part of the prepared ASD samples (containing sucrose or trehalose as a carrier forming material) were analyzed by a differential scanning calorimetry (DSC), in which a TA Q2000 calorimeter (TA instruments Co., New Castle, DE) equipped with RCS90 cooling system (TA instruments Co.) was employed. Similar to the procedures used in our previous study,[31] a few mg of the ASD sample was packed in an aluminum pan and then scanned from -50˚C to 200˚C at a rate of 10˚C/min, with an empty pan as a reference. From the obtained DSC thermogram, the exotherm heat due to the crystallization of sucrose or trehalose was determined and, by comparing them with the heats of fusion, the degrees of crystallization of the ASD sample were estimated.

3. Results and Discussion

3. 1. Optimal drying conditions for vacuum-foam- and spray-dryings

3.1. 1. Vacuum-foam-drying

As the name suggests, the foaming of a solution to be dried is essential for vacuum-foam-drying. Our previous study revealed that the timing of the needle stimulation after the initial drying was crucial for inducing the sample solution to foam at the secondary drying.[18] Hence, we measured and compared the probability for foaming of the sample solution to be induced (foaming probability) among different initial drying periods (Fig. 1(a)). When PVP was used as a carrier forming material (solid lines), the initial drying period where solution foaming was reliably induced appears to be shortened to around 30 min, compared with that for a flavor-free methanol solution of PVP.[18,19] On the other hand, in the case of disaccharides (dashed lines), foaming probability reaches approximately 100% at equal to and more than 60 min (Fig. 1(a)). The minimum initial drying period required for reliable foaming induction coincides with that for the flavor-free solution, as shown in Fig. 1(a). However, foaming probability occurs at a shorter induction period than that for a flavor-free solution (Fig. 1(a)). These findings indicate that the presence of a flavoring substance tends to reduce the time required for achieving initial drying for foaming to be induced. According to our previous study,[19] the solution viscosity needs to be increased above a certain level for foaming to be induced by needle stimulation.[14] Therefore, the flavoring substance (cinnamaldehyde) may serve to thicken the solution.

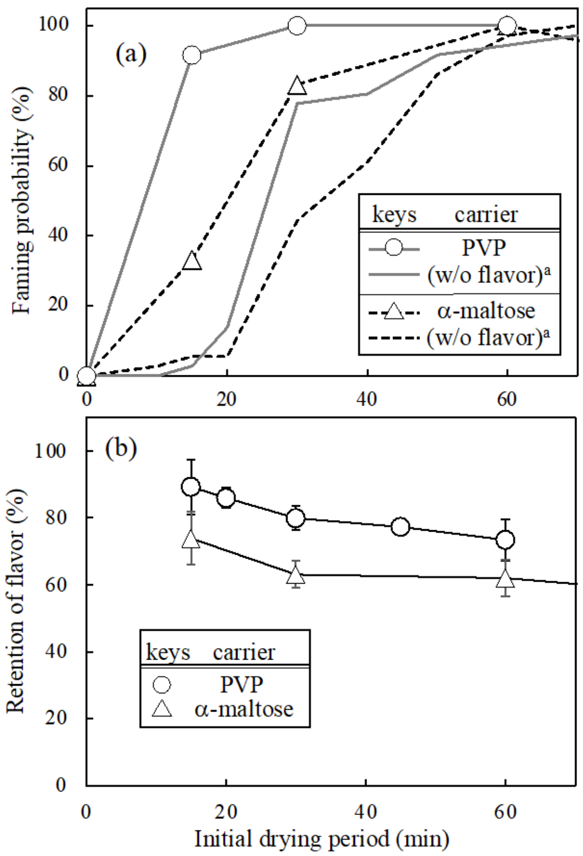


Figure 1. Probabilities of foaming induction by needle stimulation in secondary vacuum drying (a) and the retention of flavor after the secondary drying (b) as a function of the period of initial drying. Methanol and cinnamaldehyde were used as the solvent and the flavor. The initial concentrations of flavor and carrier forming material were 10 mg/mL and 100 mg/mL, respectively.

[a] Fujioka et al., 2022[19]

Flavor retention for different initial drying periods were determined for several combinations of flavor and carrier-forming material (Fig. 1(b)). As shown in Fig. 1(b), the loss of flavoring (cinnamaldehyde) after vacuum-foam-drying becomes more significant with increasing initial drying period. The α-maltose-based ASD sample showed a greater flavor loss than that for the PVP-based sample, especially when the initial drying period was up to 15 min.

Since, as shown in Fig. 1(b), flavor loss proceeds with a prolonged initial drying period, a shorter period of initial drying naturally is preferred in terms of the flavor retention. Taking these findings into account and considering the range for the initial drying period for achieving reliable foaming induction (Fig. 1(a)), the initial drying period was fixed at 20 min for PVP and 60 min for disaccharides, respectively.

3.1.2. Spray drying

A spray-drying process inherently involves multiple process parameters including inlet/outlet temperatures of the hot air, feeding flow rate, hot air flow, type/nozzle size of atomizer, as well as other issues. We examined the inlet temperature for hot air and the feeding flow rate. Figures 2(a) and (b) show the amounts of flavor (cinnamaldehyde) remaining in the spray dried sample (PVP) against the inlet hot air temperature and feed flow rate, respectively. A high flavor retention (~75%) is maintained in the range of inlet hot air temperatures from 130˚C to 160˚C. At the lower (120˚C) and higher inlet temperatures of inlet hot air (180˚C), the insufficient drying and excessive heating may cause the dried samples to collapse, respectively, resulting in comparatively low flavor retention

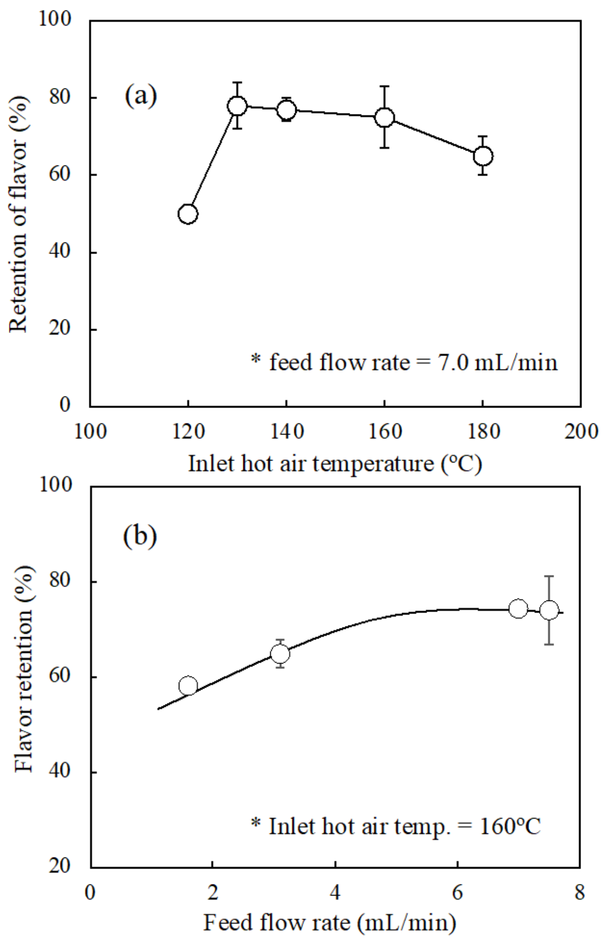


Figure 2. Retention of flavor after spray-drying as a function of (a) inlet temperature of hot air and (b) feed flow rate. As a flavor and carrier forming material, cinnamaldehyde (10 mg/mL) and PVP (100 mg/mL) were used, respectively.

(Fig. 2(a)). On the other hand, as shown in Figure 2(b), flavor retention reaches a maximum at a rate of around 6 mL/min. Based on these findings, we employed an inlet hot air temperature of 160˚C and a feeding flow rate of 7.0 mL/min for the spray-drying.

In this study, vacuum-foam-drying and conventional spray drying were employed as drying methods. On the other hand, in the research field of drying technology, various drying methods for the flavor encapsulation have been developed. Some of them, such as heat pump drying,[32,33] electrohydrodynamic drying,[34] and vacuum drying in combination with microwave,[35,36] may bring an improvement in the ASD-based flavor powderization technique. In the future study, we will explore the other drying methods in order to truly optimize the manufacturing process for the ASD of flavor.

3.2. Flavor retention after vacuum-foam- and spray-drying

In order to test the applicability of the employed drying conditions, the alcohol solution solely containing PVP or disaccharide (α-maltose or palatinose) were vacuum-foam- or spray-dried under the above-determined conditions and then analyzed for the remaining solvent amounts, the glass-transition temperatures (T_g) (Table S1) and the SEM images (Figs. S1). As shown in Table S1, the remaining solvent amounts of vacuum-foam- and spray-dried PVP were below 0.01 g-solvent/g-dry matter. On the other hand, the methanol spray-dried

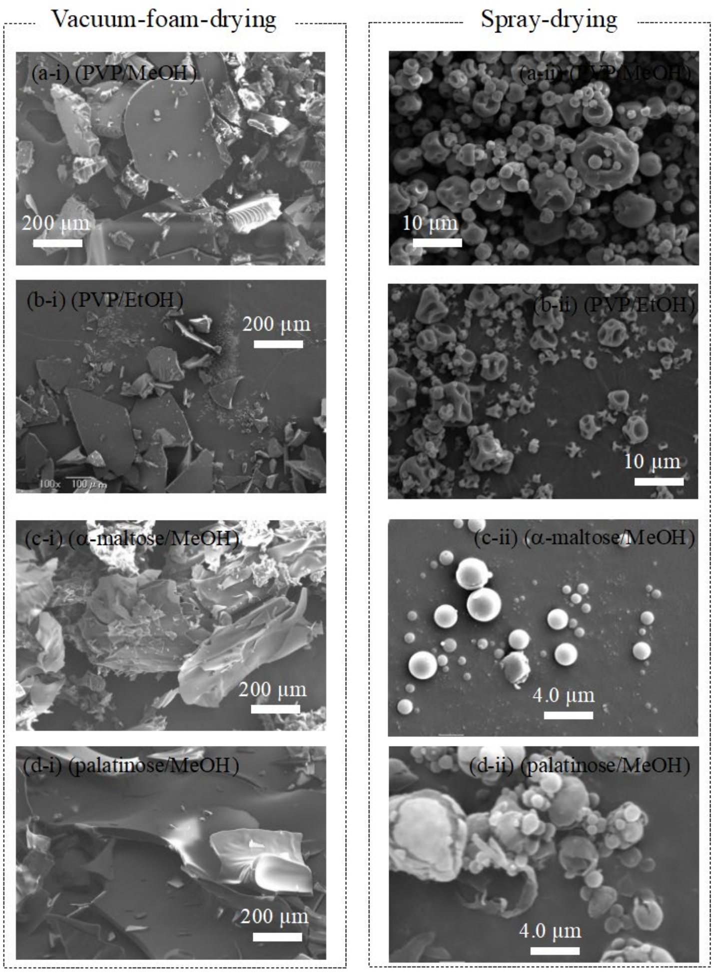


Fig. S1. SEM images of PVP (2.5k), α-maltose, and palatinose, vacuum-foam dried or spray-dried from alcohol (MeOH, EtOH) solutions. The drying conditions were those determined in this study (Sections 3.1.1 and 3.1.2)

amorphous disaccharides exhibited markedly high remaining solvent amount (0.05~0.10 g-solvent/g-dry matter), which resulted in much lower T_g values than that of the PVP alone samples (Table S1). Especially, the markedly low T_g value of the methanol spray-dried palatinose sample (-2˚C) caused the aggregation and fusion of the sample particles as shown by the SEM image (Fig. S1(d-ii)). Hence, in this study, only a limited number of the disaccharide-based ASD samples were prepared by spray-drying for the comparison.

Table S1. Remaining solvent amounts and glass-transition temperatures (T_g) of alcohol-dried amorphous PVP and disaccharides (α-maltose, palatinose) immediately after drying. The methanol or ethanol solution containing solely PVP or disaccharides were vacuum-foam- or spray dried under the conditions determined in this study (Sections 3.1 and 3.2).

solute	solvent	drying method	remaining solvent amount[a] (g/g-dry matter)	T_g (˚C)
PVP	methanol	vacuum-foam-drying	0.0070±0.0005	132±6
		spray-drying	0.0066±0.0004	131±6
	ethanol	vacuum-foam-drying	0.0091±0.0004	124±9
		spray-drying	0.0063±0.0005	125±8
α-maltose	methanol	vacuum-foam-drying	>0.002[b]	39±1[37]
		spray-drying	0.043±0.005	52±2
palatinose	methanol	vacuum-foam-drying	>0.002[b]	17±5[38]
		spray-drying	0.080±0.02	-2±2

[a] determined by measuring the mass before and after being absolutely drying in a dry oven at 130˚C for 24 h.

[b] extrapolated from the drying profiles of methanol solution containing disaccharide during vacuum-foam-drying [37]

The methanol solutions of different combinations of flavoring and carrier substances were dried under the above determined drying conditions and the degree of flavor retention was then measured (Table 2). As shown in Table 2, irrespective of the flavor type or the drying method (vacuum-foam- or spray-drying), a markedly high retention of flavor against loss during the drying process was observed when PVP was used as a carrier-forming material. Vacuum-foam-drying is superior to spray-drying in terms of flavor retention for all the combinations of flavor and carrier substances that were tested

(Table 2), probably because of the much lower sample temperature that wase used in the vacuum-foam-drying than that in the spray-drying.

In the field of pharmaceutical sciences, it has been reported that the stability of the ASD against the segregation of hydrophobic drug component is largely attributed to the miscibility between the hydrophobic drug and the carrier material.[39,40] On the other hand, considering that PVP is an amphiphilic polymer having both hydrophilic and hydrophobic moieties, a hydrophobic flavor would naturally be more miscible in amorphous PVP matrix than in amorphous sugar matrix. This is considered to be the reason for the greater flavor retention in the PVP-based ASD than in the disaccharide-based one, as shown in Table 2.

Table 2 include the results for the samples that had been emulsified and then (freeze- or spray-)dried in the presence of a carrier-forming material (α-maltose or dextran). A freeze-dried O/W emulsion sample exhibited approximately 40% retention (cinnamaldehyde) when Tween 20 and α-maltose were used as the surfactant and the

Table 2. Remaining amount of flavor (retention) after drying in the presence of different carriers. Three different drying methods, namely spray-drying (SD), vacuum-foam-drying (VFD), and freeze-drying (FD) were employed. Alternatively, the flavor oil was emulsified with the sucrose ester M-1695 and then dried in the presence of the carrier sugar. The retention values are relative values to the flavor amount before drying.

flavor	drying method	carrier	retention (%)
cinnamaldehyde	SD	PVP	74±7
		α-maltose	60±7
		palatinose	71±4
	VFD	PVP	86±5
		α-maltose	62±5 (94±5[a])
		palatinose	64±5
		trehalose	54±2
		sucrose	59±9
cinnamaldehyde (O/W emulsion)	SD	α-maltose	32±2
		dextran	33±2
	FD	α-maltose	21±5 (46±14[a])
		dextran	11±2
carvacrol	VFD	PVP	80±5
		α-maltose	56±3
		palatinose	60±6
		trehalose	51±8
		sucrose	44±4
anethole	SD	PVP	43±2
	VFD	PVP	78±5
		α-maltose	55±2
		palatinose	43±3
		trehalose	40±3
		sucrose	42±5
		maltitol	35±8
citral	SD	PVP	53±9
	VFD	PVP	81±3
		α-maltose	49±2
		palatinose	54±3
		trehalose	38±3
		sucrose	37±8
		maltitol	33±7
eugenol	SD	PVP	41±4
	VFD	PVP	78±8
		α-maltose	70±4
		palatinose	64±10
		trehalose	61±5
		sucrose	55±4
		maltitol	54±12
raspberry ketone	SD	PVP	38±6
	VFD	PVP	77±6
		α-maltose	91±9
		palatinose	95±8
		trehalose	94±14
		sucrose	92±3

[a] obtained in our previous setup [Satoh et al., 2016]

carrier forming material (Table 2). However, samples prepared using a O/W emulsion tended to be poorer in flavor retention during drying than the ASD samples.

Our previous attempt to apply ASD to flavor powderization[14] employed vacuum foam drying[18] and its superiority to the conventional emulsification-based-technique was demonstrated, which is consistent with the above-described findings. However, the measured degrees of flavor retention in the vacuum-foam-dried ASD samples in this study was frequently found to be significantly lower than those in our previous study.[14] This can be attributed to the decrease in the degree of vacuum during vacuum (foam) drying as the result of replacing the drying setup (~133 Pa (Satoh et al., 2016) → ~2,000 Pa), although the detailed mechanism responsible for this is not clear.

When different types of disaccharides were compared, α-maltose and palatinose exhibited a greater flavor retention (in vacuum foam drying) than the other two, as shown in Table 2. This can be attributed to the fact that amorphized α-maltose and palatinose are stably dissolved in methanol while trehalose and sucrose may have segregated or even partially precipitated before being dried.[38]

Table 2 indicates that anethole and citral are slightly less retained in the amorphous sugar based solid dispersions than the other flavors, probably because of their inherently high volatility (Table 1). The degree of retention of eugenol in the PVP-based ASD samples is markedly different between vacuum foaming (~78%) and spray drying (~41%), suggesting that the compatibility of eugenol with PVP may be altered slightly with the temperature used in the drying process. On the other hand, raspberry ketone was almost fully retained by the vacuum foam dried (amorphous) sugar, which is greater than in the case of the vacuum foam dried PVP-based ASD (Table 2). Judging from the high melting point of the raspberry ketone (~83˚C),[41] raspberry ketone molecules may have completely crystallized during the vacuum foam drying and thus lose its volatility, resulting in completely avoiding dissipation (Table 2).

3.3. Storage stability of vacuum foam and spray dried ASD of flavors

Figure 3 provides information on the time courses for the remaining amounts of flavoring substance in the ASD samples for the different drying method and carrier forming material. As shown in Fig. 3, the PVP-based ASDs, prepared either by vacuum-foam- or spray-drying, usually show a substantial flavor loss at the early stage of storage (1~3 days) and the dissipation then slows with further storage. The total

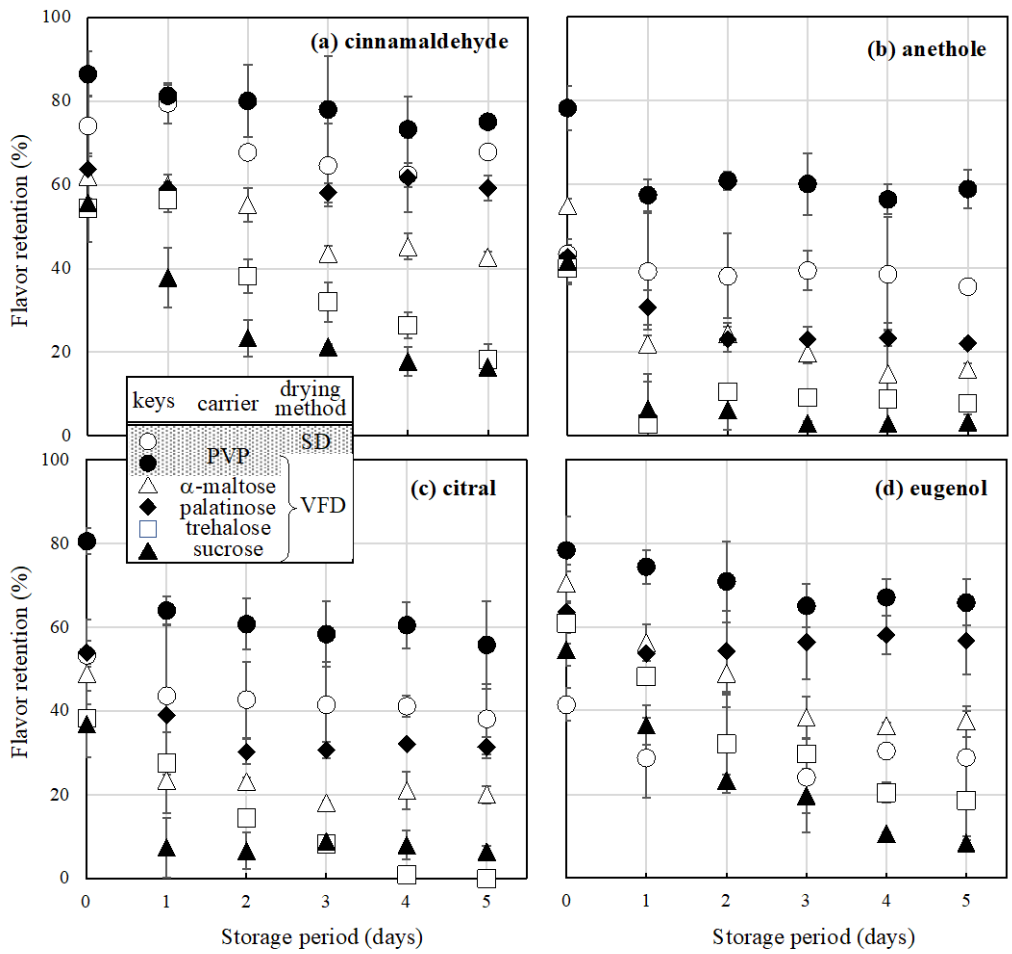


Figure 3. Loss of flavors from ASD samples during storage under a vacuum at 25°C. The initial concentrations of flavor and carrier forming material were 10 mg/mL and 100 mg/mL, respectively.

flavor loss from the PVP-based ASDs after 5-day storage is, at most, ~20% (for anethole and citral), irrespective of whether vacuum foam or spray drying were used. The vacuum foam dried α-maltose- and palatinose-based ASDs also showed a flavor loss of no more than 20% during storage (Figs. 3). These findings demonstrate that ASD-based flavor powderization is effective in terms of storage stability as well as the retention against drying.

The time course for flavor loss during storage for the sample prepared through the freeze-drying of the O/W emulsion, are also shown in Fig. S2, which shows that the amount of flavor remaining during storage is nearly constant. This indicates that the emulsification-based powderization of a flavor also has the merit of being retained during storage.

On the other hand, the sucrose- and trehalose-based ASD samples lose significant amounts of flavor during storage, as shown in Figs. 3 (closed triangles and open squares).

According to the DSC analyses, these ASD samples were found largely crystallize during storage (Fig. S3), which would result in significant flavor loss as shown in Figs. 3.

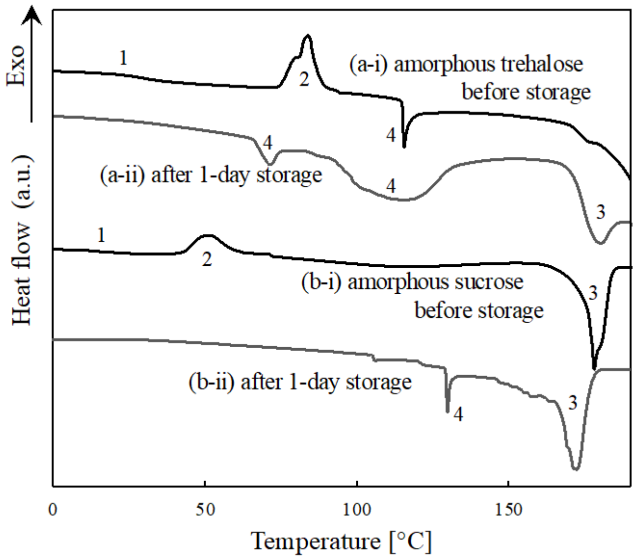


Figure S3. DSC thermograms of vacuum-foam-dried amorphous (a-i,ii) trehalose and (b-i,ii) sucrose samples, immediately after drying (solid lines) and after a 1-day storage under a vacuum (grey lines). Cinnamaldehyde was used as a flavor, at an initial concentration was 10 mg/mL. Numbers 1~3 in the graph denote the glass-to-rubber transition, crystallization, and melting of crystal respectively. The endothermic peaks, indicated by 4, likely represent the dissipation of flavor dissipation from the crystallized phase of the sugars.

3.4. Influences of flavor content and solvent type

In this study, the influence of solvent type (methanol or ethanol) and the initially amount of flavor loaded on flavor retention by the ASD-based technique was also investigated. In these investigations, PVP was used as a carrier forming material, judging from its superior flavor retention ability, as shown above. Figure 4 shows the flavor retention results (before and after 5-days of storage) for the flavor ASD samples, solvent used as well as in the flavor content. As shown in Fig. 4, the use of ethanol resulted in lower flavor retention after drying (open bars) than that of methanol, irrespective of the drying method or flavor type. This may be ascribed to the lower volatility of ethanol compared to methanol. Namely, during drying, the solvent, having a greater volatility, would evaporate more preferentially to the flavor component, resulting in greater avoidance of flavor loss.

The increase in the initial amount of flavor loaded tended to lower flavor retention at the time point immediately after spray drying (Figs. 4(a-ASD)), which is more significant when methanol is used as the solvent. In addition, regarding the vacuum foam dried ASDs (Figs. 4(a~d-VFD)), the decrease in flavor retention with increasing the initial amount of flavor loaded is less significant in the case of ethanol as a solvent than in the case of methanol. The ethanol-vacuum foam dried ASDs showed a comparatively significant loss of flavor during the drying, probably resulting in a decrease in flavor

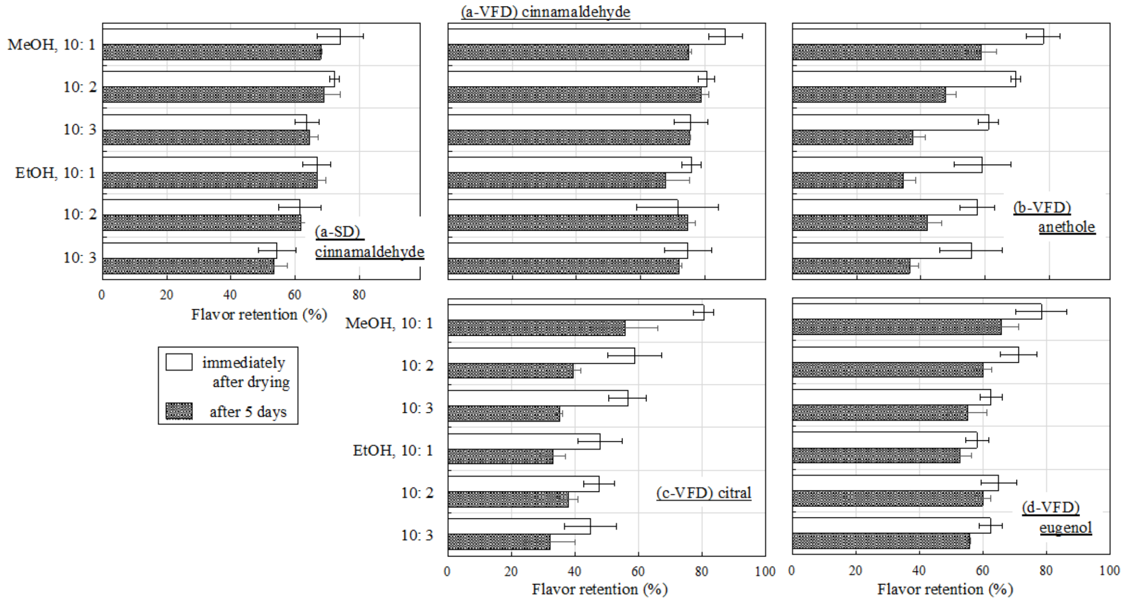


Figure 4. Retention of flavors in PVP-based ASD samples that had been dried from methanol or ethanol before (immediately after drying) and after 5-days of storage under a vacuum at 25°C. The initial concentration of flavor was 10, 20, or 30 mg/mL, the PVP concentration was 100 mg/mL. Spray- and vacuum-foam-dryings were employed as drying methods.

retention with increasing amount of initially loaded flavor relative to that for the methanol-vacuum foam dried ones (Figs. 4(a~d-VFD)).

On the other hand, storage-induced flavor loss appears to be more significant with increased drying-induced loss, as shown in Figs. 4. Namely, cinnamaldehyde shows a comparatively small loss both immediately after drying and after 5 days of storage, irrespective of spray- (Fig. 4(a-SD)) or vacuum-foam-drying (Fig. 4(a-VFD)); Whereas anethole and citral exhibit significant loss both due to vacuum foam drying and (5 days) storage, independently of the initially loaded amount and solvent type (Figs. 4(b, c-VFD)). These findings suggest that the inherent volatilities of the flavors (Table 1) are the major determinant of flavor retention during storage (Figs. 4) as well as during drying (Table 2).

4. Conclusion

This study investigated the possible use of the amorphous solid dispersion (ASD) technique for the powderization of flavoring substances, in which several types of model flavors were powderized together with a carrier forming material by vacuum foam or spray drying from methanol. Amphiphilic polyvinylpyrrolidone (PVP, MW ~24,500) and four types of disaccharides were used as model carrier forming material. Under the optimal drying conditions that were determined, ASDs of flavoring substances were prepared using different combinations and compositions of flavor and carrier substances as well as different solvents (ethanol) and their flavor retention was compared after the preparation of the sample (drying) and storage. The results demonstrate that ~~flavor loss during powderization (drying) and storage can be largely avoided when vacuum foam drying in the presence of PVP is conducted. Spray drying in the presence of PVP as a carrier substance and vacuum-foam-drying in the presence of α-maltose or palatinose also showed greater flavor retention during the drying processes and storage than O/W emulsification-based powderization. Furthermore, a more volatile flavor was more likely to be lost during drying and storage, and the flavor loss tended to be more significant with an increase in the initial amount of loaded flavor, which was more significant for methanol than for ethanol. Consequently, considering these collective findings,~~ ASD-based flavor powderization ~~appears to~~ have the potential for serving as an alternative methodology. Especially, vacuum-foam-drying in the presence of PVP could significantly prevent the flavor loss during powderization (drying) and storage.

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Figure Legends

Figure 1. Probabilities of foaming induction by needle stimulation in secondary vacuum drying (a) and the retention of flavor after the secondary drying (b) as a function of the period of initial drying. Methanol and cinnamaldehyde were used as the solvent and the flavor. The initial concentrations of flavor and carrier forming material were 10 mg/mL and 100 mg/mL, respectively.

[a] Fujioka et al., 2022[19]

Figure 2. Retention of flavor after spray-drying as a function of (a) inlet temperature of hot air and (b) feed flow rate. As a flavor and carrier forming material, cinnamaldehyde (10 mg/mL) and PVP (100 mg/mL) were used, respectively.

Figure 3. Loss of flavors from ASD samples during storage under a vacuum at 25˚C. The initial concentrations of flavor and carrier forming material were 10 mg/mL and 100 mg/mL, respectively.

Figure 4. Retention of flavors in PVP-based ASD samples that had been dried from methanol or ethanol before (immediately after drying) and after 5-days of storage under a vacuum at 25˚C. The initial concentration of flavor was 10, 20, or 30 mg/mL, the PVP concentration was 100 mg/mL. Spray- and vacuum-foam-dryings were employed as drying methods.

Fig. S1. SEM images of PVP (2.5k), a-maltose, and palatinose, vacuum-foam dried or spray-dried from alcohol (methanol, ethanol) solutions. The drying conditions were those determined in this study (Sections 3.1.1 and 3.1.2)

Figure S2. Loss of flavor (cinnamaldehyde) from samples prepared from an O/W emulsion during storage under a vacuum at 25˚C. The results for ASD samples are also shown with lines for comparison.

[a] in this study (Fig. 3(a)); [b] Satoh et al., 2016[14]

Figure S3. DSC thermograms of vacuum-foam-dried amorphous (a-i,ii) trehalose and (b-i,ii) sucrose samples, immediately after drying (solid lines) and after a 1-day storage under a vacuum (grey lines). Cinnamaldehyde was used as a flavor, at an initial concentration was 10 mg/mL. Numbers 1~3 in the graph denote the glass-to-rubber transition, crystallization, and melting of crystal respectively. The endothermic peaks, indicated by 4, likely represent the dissipation of flavor dissipation from the crystallized phase of the sugars.