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Water Solubility of Flavor Compounds Influences Formation of Flavor Inclusion Complexes from Dispersed High-Amylose Maize Starch

ONANONG TAPANAPUNNITIKUL,[†,‡] SIREE CHAISERI,[†] DEVIN G. PETERSON,[‡] AND DONALD B. THOMPSON*[,‡]

Department of Food Science and Technology, Kasetsart University, Bangkok 10900, Thailand and Department of Food Science, Food Science Building, University Park, The Pennsylvania State University, Pennsylvania 16802

High-amylose maize starch, with and without native lipid, was used to make inclusion complexes with flavor compounds to investigate the effect of water solubility of flavor compounds on inclusion complex formation. Two pairs of terpenes, having high and low water solubility, were used. Aqueous starches were dispersed by heat before adding the flavor compound. The amounts of starch, native lipid, and flavor compound in precipitates were determined, and inferences about the physical state were made using data from X-ray diffraction and differential scanning calorimetry. The water solubility of the flavor compound was related to the extent of inclusion complexation. For the higher water solubility flavor compounds, starch yield and flavor entrapment were higher, producing precipitates with the V_7 pattern. Complex formation with the low-solubility flavor compounds was most effective in the presence of native lipid, producing precipitates with the V_6 pattern. The lipid in native high-amylose maize starch may enhance complexation with low-solubility compounds by forming ternary coinclusion complexes of starch−lipid−flavor.

KEYWORDS: Flavor encapsulation, high amylose maize starch, starch inclusion complex, native starch lipid

INTRODUCTION

Inclusion complexes between starch and flavor compounds are of interest because they influence flavor perception in food and because they can lead to molecular encapsulation in flavor technology (*1*). Starch-containing matrices showed a decrease in flavor perception due to starch−flavor inclusion complex formation (*2–4*). Formation of starch−flavor complexes and the properties of the complexes have been studied for various alcohols, aldehydes, and ketones; the maximum flavor compound loading has been shown to be 4–10% (g flavor/g complex). Although flavor release is favored at high temperature (>80 °C) or upon addition of water (*5, 6*), starch−flavor inclusion complexes are poorly water-soluble, having a low flavor compound dissociation value (K_d). K_d values in the range from 2×10^{-4} to 3×10^{-3} mol/L have been reported (*7*). A lower K_d value might be useful for a slower flavor release application in food.

Starch can form inclusion complexes with a variety of compounds. Size, shape, hydrophobicity, and water solubility of the ligand contribute to the ability of the ligand to complex with starch (*1–8*). A ligand with a long-chain hydrocarbon was proposed to induce complexes of single helical amylose with the hydrocarbon included in the hydrophobic channel (*9*). It has been reported that shorter linear molecules, such as a C-8 fatty acid or a C-7 lactone, can not form stable complexes with starch (*3, 10*). Many volatile flavor compounds have a low molecular weight with a nonlinear molecular structure. Prediction of possible complex formation with starch is complicated, and it may depend on multiple structural properties. Fenchone, geraniol, menthone, and thymol have been shown to form inclusions with starch, but inclusions with cymene, citral, and cinnamaldehyde have not been observed (*8, 11*). The reason for this behavior is obscure; however, differences in their water solubility have been discussed as possible contributing factors (*8*).

In a ternary model system of a starch/monoacyl lipid/flavor compound, binding of starch−monoacyl lipid was shown to interfere with complexation of menthone. Starch−menthone inclusion complexes were reported to have lower stability but a higher binding capacity as compared to starch-monoacyl lipid (*12*). It is also well-known that the presence of native lipid interferes with starch−iodine complex formation (*13*).

Starch−flavor inclusion complex studies commonly employ amylose or lipid-free starch preparations (*3–5, 7–9, 12*). Native lipid always exists in commercial nonwaxy-type starches. To

* Corresponding author. Telephone: (814) 863-0481, fax: 814 863 6132, e-mail: dbt1@psu.edu.
[†] Kasetsart University.
[‡] Pennsylvania State University.

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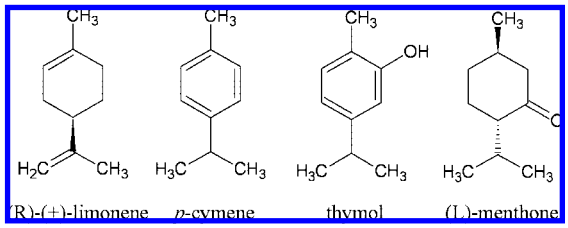

Figure 1. Molecular structure of flavor compounds.

accomplish flavor encapsulation using a commercial native starch would be of interest because the material cost would be less than for prepared amylose. With respect to the flavor compounds, water solubility of the compounds might influence their complexing ability because the concentration of free flavor compounds must be large enough for mass action to drive complex formation against the unfavorable entropic considerations. Thus, the effect of water solubility of the flavor compound on starch−flavor inclusion formation should be considered. In the present work high-amylose maize starch, with and without native lipid, was used to make inclusion complexes with flavor compounds to investigate the effect of water solubility of flavor compounds on inclusion complex formation.

MATERIALS AND METHODS

Starches. Commercial high-amylose maize starch (Hylon VII) obtained from National Starch and Chemical Company (Bridgewater, NJ) was used as the native high amylose maize starch. Lipid-free high amylose maize starch was produced by dispersing the Hylon VII with a dimethyl sulfoxide/water (90:10) mixture before precipitating with 95% ethanol, washing once with 95% ethanol and once with acetone, and drying at 40 °C overnight (*14*). The amounts of lipid in the native and the lipid-free high amylose maize starch were determined by gas chromatography (GC), after conversion of esterified and nonesterified fatty acids to fatty acid methyl esters, to be 1.06 ± 0.33 and 0.04 ± 0.01 wt% (dry basis), respectively.

Flavor Compounds. Two pairs of terpenes, having high and low water solubility but being similar in molecular weight (MW) and shape, were used to study the effect of water solubility on the starch−flavor inclusion complexes. Limonene (Aldrich, purity 98%) and cymene (Aldrich, purity 99%) were the low water-solubility flavor compounds employed; thymol (Aldrich, purity 99.5%) and menthone (Aldrich, purity 90%) were the high water-solubility flavor compounds employed. The molecular structure and properties of these flavor compounds are shown in **Figure 1**and **Table 1**.

Calculated Composition of the Mixtures Prior to Precipitation. The concentrations of dissolved flavor compounds, as calculated from the water solubilities, are shown in **Table 2**. The calculated amounts of native lipid, the weighed amounts of starch, the mole ratios of dissolved flavor compound per mole native lipid, and the moles of total ligand per mole anhydroglucose unit (AGU) of starch are also shown.

Starch−Flavor Complex Preparation. The general starch−flavor complex preparation method was adapted from methods used in previous research. Dilute aqueous starch was dispersed by heat before flavor addition (*4*). A 15 mL dispersion of native or lipid-free high-amylose maize starch (0.8% w/v) was placed in a stainless steel pressure vessel (20 mL pressure bomb, Parr Company, Moline, IL). The vessel was placed in a convection oven and was heated to 200 °C for 75 min. The vessel was removed from the oven, and the temperature was reduced with tap water before opening the vessel. The warm contents were transferred to a flask, which was placed in an 80 °C waterbath, and maintained for 15 min at 80 °C prior to flavor compound addition. Ten milliliters of dispersed starch was pipetted into a screw-cap test tube, and 0.3 mol of flavor compound/mol glucose monomer (expressed as AGU) was added to ensure an excess of added flavor. The mixtures were vortexed thoroughly, and then held at 80 °C for 1 h before cooling to room temperature in a water bath over a 6 h period. The starch−flavor compound mixtures were held at room temperature for 18 h. Wet precipitates were collected after centrifugation at 1700*g* for 15 min and then freeze-dried (Freeze mobile 12, Virtis Company, Gardiner, NY). Dispersed starches without flavor addition were used as control treatments.

Determination of Starch Precipitated by Added Flavor Compounds. The amount of starch in the precipitate was determined by an indirect method. After the precipitated starch was centrifuged (as above), the supernatant was collected for total carbohydrate analysis. The phenol−sulfuric acid assay (*22*) was used to determine the total carbohydrate. Absorbance was read at 490 nm. A standard curve was prepared using glucose standards in the range of 0–100 μg/mL. The amount of starch precipitated was calculated. The yield of dispersed starch as precipitate was also calculated. Each sample was analyzed in triplicate.

Determination of Entrapped Flavor Compounds and Lipid. Readily extracted flavor compounds and lipids of the starch−flavor compound precipitates were removed by hexane washing (*23*) before quantitative analysis of the remaining entrapped flavor compound or lipid. Each sample was analyzed in triplicate.

The entrapped flavor compound was determined by solvent extraction after starch dispersion in NaOH solution (*24*). The starch−flavor precipitates were dispersed with 1N NaOH at room temperature. Subsequent extractions with 3 mL of solvent were accomplished by stirring at room temperature for 30 min. Hexane was used to extract menthone, cymene, and limonene; diethyl ether was used to extract thymol because hexane is not a good solvent for thymol extraction. The extracts were collected, concentrated to 1 mL, and dried with Na_2SO_4 before analysis by gas chromatography. The GC (GC-HP6890) was equipped with a HP-5 column (30 m × 0.32 mm × 1 μm) and a flame ionization detector. The oven temperature regime was as follows: hold at 40 °C for 2 min, heat to 180 °C at a rate of 5 °C/min, heat to 250 °C in 2 min, and hold at 250 °C for 3 min. 2-Decanone was used as internal standard.

Entrapped lipid was estimated after methylation of all fatty acid chains with MeOH/H_2SO_4 (98/2, v/v) (*9*). A 150 μL portion of heptanoic acid in toluene (1 mg/mL) was used as an internal standard. The methylated-FFA was extracted with 5 mL of hexane at room temperature for 1 h. The solvent was concentrated to 1 mL and dried with Na_2SO_4 before analysis by GC. The oven temperature regime was as follows: hold at 150 °C 2 min, heat to 250 at 35 °C/min, and hold at 250 °C for 3 min. Methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, and methyl linolenate were used as standards for quantitation.

Wide Angle X-ray Diffractometry (XRD). XRD analyses were conducted using an X-ray diffractometer (Philips X'Pert-MRD) and Philips X'Pert-MRD software version 4.0 for data collection. Moisture content of the samples was between 5.9 and 6.6%. The samples were analyzed between 4° and 30° 2θ at a step size of 0.02°, a scan speed of 2.0 s/step, at a tension of 40 kV, and a current of 45 mA. Each sample was analyzed in duplicate.

Differential Scanning Calorimetry (DSC). The wet precipitates were used for DSC investigation. Excess associated supernatant was removed by blotting the precipitates (Micro wipe). Approximately 80 mg of blotted precipitate (~80% g solid/g total) was weighed into a stainless steel DSC pan. A differential scanning calorimeter (Perkin-Elmer DSC-7) was used. The pan was held at 0 °C for 1 min, then heated to 180 at 10 °C/min, cooled from 180 to 0 °C at 10 °C/min, and reheated from 0 to 180 °C at 10 °C/min. After DSC analysis, the moisture content of each sample was determined by breaking the seal on the pan and drying at 130 °C for 2 h. The moisture content of samples was between 77 and 87%. Each sample was analyzed in triplicate.

RESULTS

Determination of Starch Precipitated by Added Flavor Compounds. The starch yields, expressed as precipitated starch relative to initially dispersed starch, are reported in **Table 3**. Without flavor compound addition, more than 10% of the native starch precipitated, but the lipid-free starch precipitated only slightly under the same conditions (less than 1%). In the

Table 1. Properties of the Flavor Compounds

terpene	chemical formula	molecular weight (g/mol)	odor[a]	hydrophobicity[b]	water solubility[c] (mg/l)
(*R*)-(+)-limonene	$C_{10}H_{16}$	136.2	lemon	4.35	1.1×10^1
p-cymene	$C_{10}H_{14}$	134.2	strong carrot	3.34	5.1×10^1
thymol	$C_{10}H_{14}O$	150.2	sweet, medicinal	3.89	5.6×10^2
(−)-menthone	$C_{10}H_{18}O$	154.3	\menthol	2.83	6.9×10^2

[a] Reference *15*. [b] Expressed as log *P*, where *P* = partition coefficient between octanol and water, from refs *16*−*18*. [c] References *18*−*21*.

Table 2. The Amount of Added Flavor Compounds, Dissolved Flavor Compounds, Native Lipid, and Starch Prior to Precipitation

flavor compound	flavor compound[a]				dissolved flavor	dissolved flavor
	added flavor[b], (mmol)	dissolved flavor[c], (mmol)	native lipid[d], (mmol)	starch[e], (mmol)	native lipid, (mol/mol)	starch AGU, (mol/mol)
none			3.0×10^{-3}	4.9×10^{-1}		
limonene	1.50×10^{-1}	7.3×10^{-4}	3.0×10^{-3}	4.9×10^{-1}	0.24	1.5×10^{-3}
cymene	1.50×10^{-1}	3.8×10^{-3}	3.0×10^{-3}	4.9×10^{-1}	1.22	7.8×10^{-3}
thymol	1.50×10^{-1}	3.8×10^{-2}	3.0×10^{-3}	4.9×10^{-1}	12.0	7.7×10^{-2}
menthone	1.50×10^{-1}	4.5×10^{-2}	3.0×10^{-3}	4.9×10^{-1}	14.6	9.3×10^{-2}

[a] The amount in a 10 mL dispersion. [b] To give equal mols of added flavor compound. [c] Calculated based on water solubility of the flavor compound. [d] Calculated from lipid content of high-amylose maize starch. [e] Calculated from weight of dry starch added, expressed as mmol starch AGU.

Table 3. The Yield of Starch in Precipitation

high-amylose maize starch	flavor compound added	% starch yield[a]
with native lipid	none	11.5 ± 0.2 B
	limonene	11.0 ± 1.8 B
	cymene	11.8 ± 0.4 B
	thymol	39.5 ± 7.8 E
	menthone	44.0 ± 11.9 E
lipid-free	none	0.9 ± 0.4 A
	limonene	0.3 ± 0.6 A
	cymene	0.5 ± 0.9 A
	thymol	21.0 ± 7.9 C
	menthone	29.3 ± 6.0 D

[a] Means with the same letter superscript are not significantly different, at P < 0.05, as determined using Duncan's multiple range test. [(precipitated starch)/(dispersed starch)] × 100. Mean and standard deviation, $n = 6$.

presence of added low-solubility flavor compounds, the native starch precipitated in essentially the same yield as without flavor addition (~11%). In the presence of low-solubility compounds, lipid-free starch yielded little precipitate, similar to what was observed without flavor compound addition (<1%). In the presence of high-solubility flavor compounds, for both native starch and lipid-free starch, starch yields were considerably greater. The maximum starch yields were achieved by precipitation of native starch with the highest solubility flavor compound, menthone (44%).

Determination of Entrapped Flavor Compounds and Lipid. The amount of entrapped flavor compound and lipid in solvent-washed starch precipitates and the yield of entrapped flavor compound and lipid are reported in **Table 4**.

With native starch, the proportion of entrapped flavor compounds in precipitates differed greatly for low and high-solubility compound addition (**Table 4**). Limonene and cymene were entrapped only at about 1%, whereas thymol and menthone were entrapped at 5.8 and 10.4%, respectively. The lipid-free starch entrapment of flavor compounds was only observed for precipitates with a high-solubility flavor compound (10.5 and 7.6% for thymol and menthone, respectively).

The maximum lipid entrapment was observed for precipitation of native starch without any addition of flavor compound (6.7%). Addition of a high water-solubility flavor compound led to appreciably less entrapped lipid than did addition of a low water-solubility flavor compound.

In the presence of native lipid, a higher yield of flavor compounds tended to occur than in the absence of native lipid. Only in the presence of native lipid was any appreciable yield of limonene and cymene observed (73.9 and 17.8%, respectively). The lipid yield decreased with the addition of any of the flavor compounds.

The Stoichiometry of Entrapped Flavor Compounds and Lipid. The ratio of precipitated starch to precipitated flavor compound after solvent-washing was calculated by first subtracting the lipid−associated starch from the total precipitated starch; the stoichiometry is expressed as moles of glucose monomer (AGU) in starch per mole flavor compound. Results are reported in **Table 5**. The addition of high-solubility flavor compounds led to a similar mole ratio of starch AGU to flavor compound for both native starch and lipid-free starch, at about 10 mol starch AGU per mole flavor compound. The low-solubility flavor compounds show a higher ratio than the high-solubility flavor compounds, at 52 and 27 starch AGU per mole limonene and cymene, respectively.

XRD Pattern. X-ray diffractograms of washed starch−flavor compound precipitates are shown in **Figure 2**. For native high-amylose maize starch, precipitates that formed without flavor compound addition showed the same XRD pattern as those that formed with addition of limonene and cymene. The reflections at 13.7 and 20.6° 2θ indicate the characteristic V_6 pattern (*12*). In contrast, with the addition of high-solubility flavor compounds (thymol and menthone) the reflections at 7.1, 12.9, and 17.8° 2θ correspond to the V_7 pattern (*12, 25*). When the high-solubility flavor compounds were added to either lipid-free starch or native starch, precipitates had a predominant V_7 pattern. We evaluated the effect of adding 1% lysophosphatidyl choline on the formation of flavor compounds with defatted starch, and we observed X-ray diffractograms with a V_7 pattern for menthone and thymol but with a V_6 pattern for limonene and cymene (data not shown), consistent with diffractograms labeled 4 and 5 in **Figure 2**.

DSC. Upon initial heating (**Figure 3a**), native starch precipitated without flavor addition showed endotherms with two peaks, with T_p at about 100 and 112 °C. For native starch precipitated with the low-solubility flavor compounds limonene or cymene, the higher-temperature T_p values predominated, but at a lower T, at about 102 and 100 °C, respectively. The lower-T peak, observed more as a prominent leading shoulder, was also

Table 4. Flavor Compound and Lipid Entrapment in Solvent-washed Precipitates[a,b]

		entrapment[c] (%)		yield (%)	
high-amylose maize starch	flavor compound	entrapped flavor	entrapped lipid	flavor compound[d]	lipid[e]
with native lipid	none	ND	6.7 ± 1.7 C	ND	72.9 ± 18.9
	limonene	0.8 ± 0.0 A	3.5 ± 0.9 AB	73.9 ± 0.2	38.7 ± 9.5
	cymene	1.0 ± 0.0 A	5.1 ± 1.5 B	17.8 ± 0.8	59.8 ± 16.6
	thymol	5.8 ± 0.3 B	0.9 ± 0.5 A	32.2 ± 1.4	36.7 ± 17.4
	menthone	10.4 ± 2.7 C	1.0 ± 0.2 A	52.3 ± 13.7	42.3 ± 10.2
lipid-free	none	IP	IP	IP	IP
	limonene	IP	IP	IP	IP
	cymene	IP	IP	IP	IP
	thymol	10.5 ± 0.5 C	ND	31.3 ± 1.6	ND
	menthone	7.6 ± 3.0 B	ND	25.6 ± 10.0	ND

[a] Mean values with standard deviations, $n = 3$. IP = insufficient precipitate for analysis; less than 1% starch yield; ND = not determined. Means in the same column with the same letter superscript are not significantly different, at $P < 0.05$, as determined using Duncan's Multiple Range Test. [b] Lipid is estimated as fatty acid content. [c] (g of compound in precipitate/g precipitate dry weight) × 100. [d] (Weight of flavor compound in precipitate/weight of calculated dissolved flavor compound) × 100. [e] (Weight of lipid in precipitate/weight of native lipid) × 100.

Table 5. Stoichiometry of Starch−Flavor Compound Complexes[a,b]

high-amylose maize starch	flavor compound	precipitated starch, (mmol AGU)	entrapped lipid, (mmol)	entrapped flavor, (mmol)	starch associated with lipid, (mmol AGU)	starch not associated with lipid, (mmol AGU)	starch not associated with lipid/mol of flavor compound, (mol AGU/mol flavor compound)
with native lipid	none	57 ± 2	2.5 ± 0.7	ND	57		
	limonene	55 ± 11	1.1 ± 0.3	0.54 ± 0.00	26	28	52
	cymene	58 ± 3	1.8 ± 0.6	0.68 ± 0.03	40	18	27
	thymol	195 ± 48	1.3 ± 0.6	12.1 ± 0.6	28	166	14
	menthone	217 ± 73	1.3 ± 0.4	23.7 ± 6.1	28	189	8
lipid-free	none	IP	IP	IP	IP	IP	IP
	limonene	IP	IP	IP	IP	IP	IP
	cymene	IP	IP	IP	IP	IP	IP
	thymol	104 ± 49	ND	11.8 ± 0.6	ND	104	9
	menthone	145 ± 37	ND	11.6 ± 4.5	ND	145	12

[a] The amount in washed precipitates from a 10 mL dispersion. [b] IP = insufficient precipitate for analysis; less than 1% starch yield; ND = not determined.

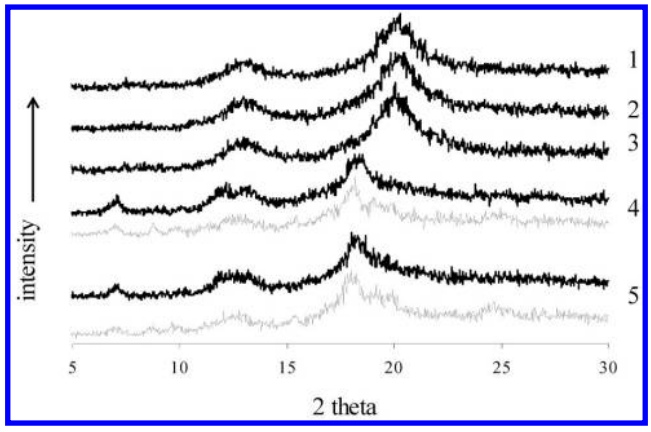

Figure 2. The XRD diffractogram of washed starch−flavor compound complexes: black lines, native starch; light-grey lines, lipid-free starch. (1) no flavor compound added, (2) with limonene, (3) with cymene, (4) with thymol, and (5) with menthone.

observed for both limonene and cymene samples (with T_{onset} about 60 °C for each). For native starch precipitation with high-solubility flavor compounds thymol or menthone, the thermograms had less of the leading shoulder. The endotherm for native starch−thymol has a two-component peak (with a second component T_p of 103 °C), whereas native starch−menthone has only one broad peak (with T_p 119 °C).

Upon cooling (**Figure 3b**), native starch precipitated without flavor compound addition showed an exothermic amylose−lipid complexation peak at $T_p \approx 67$ °C and also showed a broad exothermic peak at $T_p \approx 20$ °C, attributed to amylose association (*26*). With the addition of low-solubility flavor compounds limonene and cymene, native starch precipitates also showed an initial exothermic peak, with $T_p \approx 67$ °C, but an amylose association peak was observed only for the addition of limonene to the native starch. With the addition of high-solubility flavor compounds, the thermograms showed no evidence of an amylose−lipid exothermic peak at the usual temperature. Exothermic peaks were observed at $T_p \approx 24$ and 45 °C, which might be attributed to amylose−thymol and amylose−menthone, respectively.

Upon reheating of native starch without added flavor compound (**Figure 3c**), only one peak was observed, corresponding to the amylose−lipid complex. With the addition of a flavor compound, either a leading shoulder or two peaks were observed.

When the high-solubility flavor compounds menthone and thymol were added to lipid-free starch precipitates (recall that little or no precipitate occurred with no flavor compound or with the low-solubility flavor compounds), thermograms for initial heating and cooling were not much different, respectively, than for when these compounds were added to the native starch, as they still primarily exhibited a single event (**Figure 4**). However, upon reheating (**Figure 4c**), the lipid-free starch preparations showed only one endothermic peak, whereas native starch preparations showed two endothermic peaks.

DISCUSSION

Precipitation of starch might result from three straightforward mechanisms. First of all, simple retrogradation can lead to starch precipitation under certain conditions. However, the lipid-free starch without added flavor compound led to no appreciable

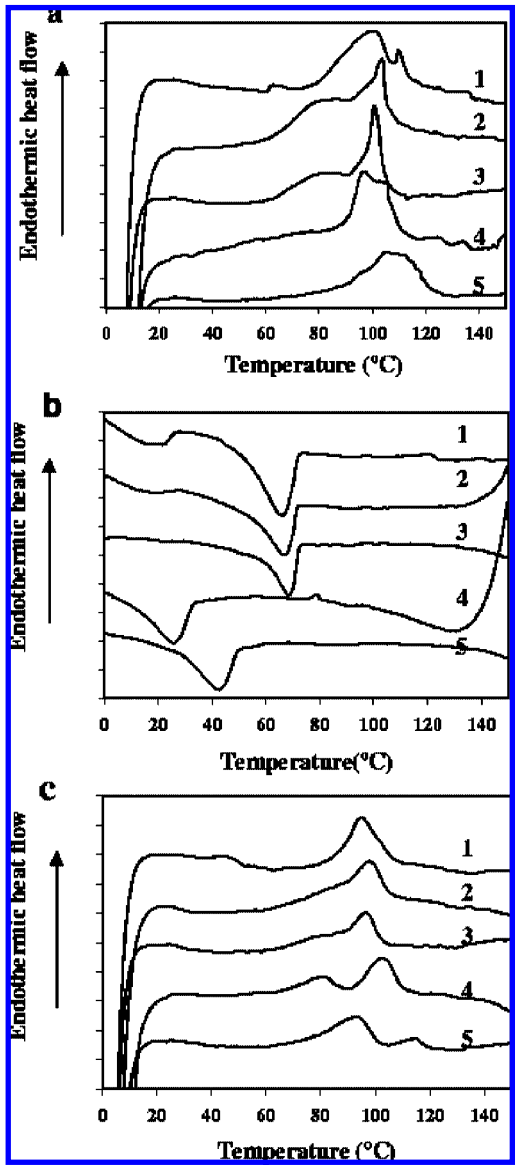

Figure 3. DSC thermograms of native starch−flavor complexes; (**a**) initial heating, (**b**) cooling, and (**c**) reheating. (1) no flavor compound added, (2) with limonene, (3) with cymene, (4) with thymol, and (5) with menthone. Unwashed precipitates were analyzed at 70–80% solids after removing excess supernatant (see Materials and Methods section). Most flavor compounds not associated with starch were removed by decanting supernatant and then blotting the precipitates.

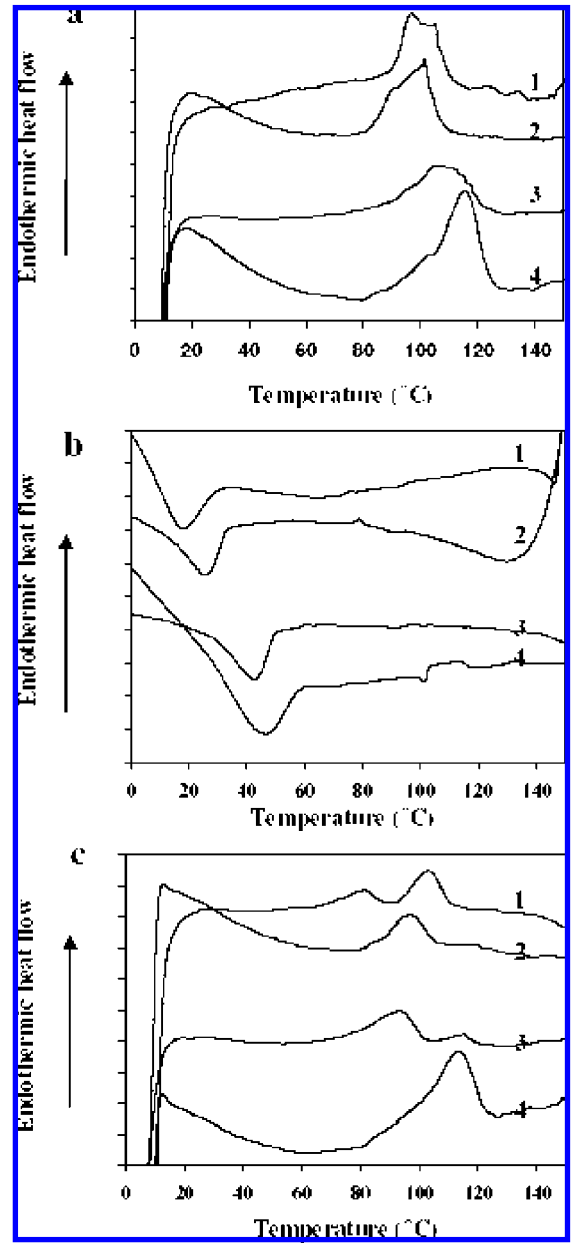

Figure 4. DSC thermograms of lipid-free starch−flavor complexes, with native starch−flavor complexes shown for comparison; (**a**) initial heating, (**b**) cooling, and (**c**) reheating; (1) native starch with thymol, (2) lipid-free starch with thymol, (3) native starch with menthone, and (4) lipid-free starch with menthone.

precipitation in the conditions of our work. Furthermore, there was no evidence of the B pattern by X-ray diffraction for any of the precipitates examined, thus providing no evidence of retrogradation. Second, starch−monoacyl lipid complexes are known to precipitate under certain conditions. For the native starch without added flavor compound, over 10% of the starch precipitated by this mechanism, as the V_6 pattern was confirmed by X-ray diffraction. Third, starch-flavor compounds may form complexes and precipitate. In the present work, the high water-solubility compounds thymol and menthone led to appreciable precipitate with lipid-free starch, as the V_7 pattern confirmed. However, the low water-solubility flavor compounds limonene and cymene had no such effect on the lipid-free starch. For starch and flavor compounds in the presence of native lipid, two important observations could be made: (1) more starch precipitated with the addition of the high water-solubility flavor compounds than with the low water-solubility flavor compounds, and (2) appreciably more starch precipitated with the addition of low water-solubility flavor compounds than did so without native lipid. An apparent three-way interaction of starch, native lipid, and flavor compound is discussed below.

Water Solubility of Flavor Compounds Directly Affects Starch−Flavor Inclusion Complex Formation and Precipitation. To eliminate a possible competitive effect of native lipid, lipid-free starch was used to make complexes with the four flavor compounds. The low-solubility flavor compounds (limonene and cymene) added to lipid-free starch did not lead to any appreciable precipitation; however, the high-solubility flavor compounds (thymol and menthone) led to starch precipitates. The low-solubility compounds might have been expected to readily form inclusion complexes because of the similar or greater hydrophobicity as compared to the high-solubility flavor

compounds (see **Table 1**), because the hydrophobic nature of the ligand molecule is believed to induce single-helix amylose (*7, 11*). Although positive cooperativity of amylose inclusion complexation has been reported (*27*), the low water solubility dictated that only a relatively small amount of dissolved limonene and cymene (1×10^{-3} and 8×10^{-3} mol per mole AGU starch) was possible. With less available ligand, shorter, inherently less stable complexes become more likely. The decreased stability of individual complexes would reduce the probability of subsequent nucleation, limiting crystallization and precipitation. By this reasoning, when the high-solubility flavor compounds were added, the likelihood of stable complexation followed by nucleation and precipitation would be enhanced.

The Effect of Native Lipid on Starch−Flavor Inclusion Complexation and Precipitation. In the presence of starch with native lipid, the addition of low-solubility flavor compounds led to formation of precipitates that included both lipid and the low-solubility flavor compound. Moreover, for these compounds higher starch and flavor yield were achieved with native starch as compared to lipid-free starch.

The native lipid apparently plays an important role in precipitation of the low-solubility flavor compounds. Native lipid in high-amylose starch is monoacyl, composed of free fatty acids and lysophopholipids; the fatty acid composition (free and in phospholipids) is primarily palmitic acid, stearic acid, and linoleic acid (*28*). Each of these fatty acids has a long enough hydrocarbon chain to form stable inclusion complexes with starch (*9*). Helical inclusion complexation involving native starch and flavor compound might be viewed as a ternary system of starch, native lipid, and flavor compound. The lipid would form an inclusion complex with starch more avidly than with the flavor compound because K_d for lipid complexes is lower (*12*) (values have been reported to be 4.27×10^{-5} and 4.93×10^{-4} mol/L for monostearate and menthone, respectively). A stable amylose−lipid complex could influence amylose chains to form a more extended complex with either the flavor compound or the lipid molecule as the included molecule. In the system of starch with native lipid and added low-solubility flavor compound, starch precipitation may consist of two types of inclusion complex: starch−lipid and starch−lipid−flavor compound. Starch−lipid complexes are undoubtedly important when native starch is precipitated with low-solubility flavor compounds because the V_6 XRD pattern was observed. The V_6 pattern is well-known for amylose inclusion complexes with monoacyl molecules; the V_6 pattern describes a helix with 6 AGU per turn (*29*). The entrapped limonene and cymene were 0.8 and 1.0% respectively, indicating substantial amounts of flavor compound were also present. Thermal analysis suggests the existence of a coinclusion complex of starch−lipid−flavor compound, as the DSC heating thermogram has a leading shoulder (**Figure 3**). Coinclusion complexes could be nonhomogeneous in helical structure, a mixture of 6 and 7 AGU per turn. A mixed helical structure would result in less stability of the complexes.

With high-solubility flavor compounds added to native starch, three types of inclusion complexes may exist: starch−flavor compound, starch−lipid, and starch−lipid−flavor compound. Because the molar concentration of the flavor compound is much higher as compared to the native lipid (before precipitation, the mole ratios of flavor/lipid are 12.0 and 14.6 for thymol and menthone, respectively), the major complexes may be the starch−flavor compound complexes. The observed V_7 XRD pattern favors this interpretation because the V_7 pattern is believed to describe an amylose inclusion complex with a bulkier molecule such as a branched or a cyclic molecule, with 7 AGU starch per helical turn (*30*). The multiple-component endotherm upon reheating (**Figure 4**) suggests that some starch−lipid or starch−lipid−flavor compound complexes may exist even though only a small amount of entrapped lipid was found (about 1%, **Table 4**). Thermal analysis can not distinguish between starch−flavor compound and starch−lipid because of the similar T_p values of starch−flavor and starch−lipid (**Figure 3**).

In the literature, analysis of a ternary model system of starch, monoacylglyceride, and flavor has been reported. In that work, binding of monoacylglyceride strongly inhibited the inclusion of flavor compound, whereas flavor compound binding had little effect on the binding of monoacylglyceride (*12*). The present report did show some competitive effect of native lipid and flavor molecules. In the presence of any flavor molecule, the native lipid had less ability to form inclusion complexes with starch, as shown by the lower lipid yield. However, in the presence of native lipid, a synergistic effect on flavor inclusion complexation was also observed, as shown by the increase in the flavor entrapment and flavor yield (**Table 4**).

We considered that the decreased lipid yield in the presence of the flavor compound even as flavor yield increased may be due to the behavior of lysophosphatidylcholine, the major monoacyl lipid type in the native starch. Some lysophosphatidylcholine may form micelles in the starch−flavor compound mixture, resulting in a decreased amount of molecularly dispersed lipid for inclusion. At the same time, the long hydrocarbon chain of the lysophosphatidylcholine that was molecularly dispersed may promote the stability of starch−flavor inclusion helices of the V_7 type (perhaps as mixed complexes of lysophosphatidylcholine/flavor compound), resulting in an increase in both starch yield and flavor yield (**Tables 3** and **4**).

The stoichiometry of starch complexes with the high-solubility flavor compounds was similar to that described in a previous report that found about 10 starch AGU per mole flavor compound (*6*). The low-water solubility flavor compounds had a far larger molar ratio of AGU per flavor compound (**Table 5**). This higher ratio indicates that, under these conditions, appreciable precipitated starch was not complexed with either native lipid or a low-solubility flavor compound. The precipitate of starch−lipid−low-solubility flavor compounds had less thermal stability as compared to the coinclusion complexes of high-solubility flavor compounds, as lower-temperature melting endotherms were observed (**Figure 3a**). Existence of starch not associated with lipid and flavor compound is consistent with our observation of a broad exothermic peak for amylose association at $T_p \approx 20$ °C (**Figure 3b**). We have no evidence to infer whether this portion of the starch is random coil or single-helical, because either is consistent with the evidence, but the lack of any indication of a B pattern suggests that it is unlikely to be double helical.

Flavor loading using high-amylose maize starch in the present report may be compared to other reports using cyclodextrin to make flavor complexes (*31, 32*). There has been no description of cyclodextrin inclusion complexes for cymene, thymol, and menthone. For various other flavor compounds, flavor loading in the range 5–10% was reported (*31*). Limonene complexes with cyclodextrin had flavor loading of about 10% (*32*). In the present report, starch−thymol and starch−menthone complexes have flavor loading of about 5–10% (**Table 4**). For limonene and cymene added to native high-amylose maize starch, flavor loading was only about one tenth as great (**Table 4**).

We conclude that water solubility of flavor compounds influences the extent of complexation with high-amylose maize starch; the higher-water-solubility flavor compounds led to greater precipitated starch yield and also to more flavor entrapment. The native high-amylose maize starch, but not the lipid-free starch, can form inclusion complexes with flavor compound of both low and high water solubility. The presence of native lipid increased the extent of precipitated starch yield, flavor entrapment, and flavor yield for the four flavor compounds tested. Three types of complexes may be involved: starch−flavor compound, starch−lipid, and starch−lipid−flavor compound. Besides the lower raw material cost, the use of native high-amylose maize starch for flavor encapsulation by this inclusion technique has the advantage of effectively entrapping low-solubility flavor compounds that are not entrapped without the presence of native lipid. The impact of native lipid of high-amylose maize starch on flavor release and flavor quality remains to be explored.

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