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Volatile Release from Whey Protein Isolate–Pectin Multilayer Stabilized Emulsions: Effect of pH, Salt, and Artificial Salivas

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ABSTRACT: Whey protein isolate (WPI) and pectin can form a multilayer at the oil–water interface when they are oppositely charged. In this study, effects of pH, salt, and artificial salivas on emulsion stability and volatile release from multilayer emulsions were investigated. Results showed that emulsions (0.5 wt % WPI, 10 wt % oil) with pectin content ≤0.1 wt % had rapid phase separation at pH 4 and 5, and emulsions with higher pectin content (≥0.2 wt %) had good stability. Due to an electrostatic screening effect, multilayer emulsions collapsed when subjected to ≥150 mM NaCl solutions at pH 5. When diluted with artificial salivas containing salts, mucin, and/or α-amylase, multilayer emulsions showed rapid droplet aggregation. GC headspace analysis found that volatiles had significantly lower initial headspace concentration ($C_{initial}$) in multilayer emulsions, and the $C_{initial}$ correlated negatively with pectin content in emulsions. Emulsions at pH 7 had more volatiles released to the headspace than emulsions at pH 5. However, changes in pectin content and pH did not show a significant effect on release rate of most volatile compounds. In salt-treated multilayer emulsions, $C_{initial}$ and release rates of volatiles increased with NaCl content. Addition of salivas triggered higher release of hydrophobic volatiles and lower release of hydrophilic volatiles, which was mostly due to dilution effect and saliva-induced emulsion instability.

KEYWORDS: *multilayer emulsion, pectin, WPI, stability, volatile release, artificial saliva*

INTRODUCTION

A big majority of foods exist as emulsions, either partly or wholly, such as milk, butter, and orange juice. Emulsions consist of two immiscible phases, one of which is dispersed in the other as small droplets. Studies on emulsions as delivery systems to protect, solubilize, and control the release of bioactive compounds have been well reviewed.[1,2] Volatile flavor compounds make large contributions to the organoleptic properties of foods. Volatile release from an emulsion involves the partitioning and mass transfer of the volatile molecules among oil phase, interface, water phase, and finally headspace.[3] Change in headspace concentration and release rate could affect flavor perception. Successful development of delivery systems with controlled volatile release depends on a good understanding of the effects of emulsion properties (e.g., droplet size, viscosity) and of environmental stresses on volatile release, as well as the interaction between volatile compounds and emulsion components.

Emulsions are thermodynamically unstable systems and are prone to destabilize (e.g., coalescence, creaming) during food processing, transportation, or storage. In a destabilized emulsion, volatiles can release in undesirable ways. Emulsion stability against environmental stresses can be improved by strengthening the mechanical properties of the interfacial layer by multilayer adsorption at the interface.[4] Multilayer emulsions are generally prepared through a layer-by-layer (LBL) electrostatic deposition technique, which consists of two (or more) steps of layer formation. A charged emulsifier (e.g., SDS, lecithin, protein) is first deposited onto the droplet surface during emulsification; then an oppositely charged emulsifier or polymer (e.g., protein, polysaccharide) is introduced and attracted by the previously adsorbed layer, forming a second layer. Emulsions containing oil droplets surrounded by multilayered interface have been reported to have better stability against pH change, heating, freeze–thawing cycling, etc.[5−7] Furthermore, in a multilayer emulsion the outer layer can be detached from oil droplets by changing pH, salt concentration, or temperature, thereby providing variable encapsulation capacities in response to different environmental triggers.[5,8] Encapsulations of β-carotene, lemon/orange oil, and fish oil in multilayer emulsions have been investigated.[9−11]

Volatile compounds in food systems are usually more active and sensitive to environmental changes, and multilayer emulsions can also be used to mediate their release behavior. In β-lactoglobulin–pectin stabilized emulsions, lipophilic volatiles could be released at lower rates over wide pH and salt concentration ranges.[12] When the emulsion was placed in a model mouth, the pectin layer hindered the release of lipophilic volatiles.[13] By increasing the concentration of ingredients forming the multilayer to a certain level, headspace concentration of volatile compound could also be reduced.[11] When the multilayer emulsion was spray-dried, more flavor retention could be obtained.[14] The main advantages of this type of emulsion are that the multilayer could slow volatile molecule movement across the oil–water interface due to enhanced hindrance effect and that the complex layer may adsorb more volatiles. Moreover, the delayed volatile release could disappear or be weakened when the outer layer is detached. However, literature studies put less emphasis on the linkage between

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Table 1. Constituents and Concentrations of Artificial Salivas Used in the Study[a]

S1	S2	S3	S4	S5
deionized water	10 mL KCl 89.6 g/L	10 mL KCl 89.6 g/L	10 mL KCl 89.6 g/L	10 mL KCl 89.6 g/L
	10 mL NaSCN 17 g/L	10 mL NaSCN 17 g/L	10 mL NaSCN 17 g/L	10 mL NaSCN 17 g/L
	10 mL KH_2PO_4 100.6 g/L	10 mL KH_2PO_4 100.6 g/L	10 mL KH_2PO_4 100.6 g/L	10 mL KH_2PO_4 100.6 g/L
	10 mL Na_2SO_4 129.33 g/L	10 mL Na_2SO_4 129.33 g/L	10 mL Na_2SO_4 129.33 g/L	10 mL Na_2SO_4 129.33 g/L
	20 mL $NaHCO_3$ 84.7 g/L	20 mL $NaHCO_3$ 84.7 g/L	20 mL $NaHCO_3$ g84.7 /L	20 mL $NaHCO_3$ 84.7 g/L
	1.7 mL NaCl 175.3 g/L	1.7 mL NaCl 175.3 g/L	1.7 mL NaCl 175.3 g/L	1.7 mL NaCl 175.3 g/L
	5 mL $CaCl_2$ 22.2 g/L	5 mL $CaCl_2$ 22.2 g/L	5 mL $CaCl_2$ 22.2 g/L	5 mL $CaCl_2$ 22.2 g/L
	8 mL urea 25 g/L	8 mL urea 25 g/L	8 mL urea 25 g/L	8 mL urea 25 g/L
		25 mg mucin	20 mg α-amylase	25 mg mucin
				20 mg α-amylase

[a]All solutions were prepared with deionized water and used after centrifugation.

structural change of the emulsion and volatile release, particularly at adverse environmental conditions, and more profound work is required to understand the mechanism.

In the current study, model whey protein isolate (WPI)−pectin stabilized multilayer emulsions were designed with different interfacial structures suitable for volatile delivery, and the main objective was to study the release behavior of volatile compounds in these mulatilayer emulsions under broad environmental conditions. Emulsions were subjected to different pH values, NaCl concentrations, and artificial salivas. Emulsion properties and release behavior of volatile compounds were further investigated and correlated to understand the environmental effects. The knowledge obtained from this study may assist the development of novel foods with desired flavor profiles via emulsion structural modification.

MATERIALS AND METHODS

Materials. Apple pectin (degree of esterification, 70−75%; molecular weight, 30−100 kDa) was purchased from Sigma-Aldrich (St. Louis, MO, USA). WPI (BiPro), which contained 71% β-lactoglobulin and 12% α-lactalbumin, was kindly offered by Davisco Food International (Le Sueur, MN, USA). Sunflower oil was purchased from a local supermarket and used without further purification. 1-Propanol (>99.5% purity), diacetyl (butane-2,3-dione, >99.5% purity), 2-pentanone (>99% purity), ethyl octanoate (>99% purity), and 2-heptanone (>99% purity) were all products of Sigma-Aldrich. Analytical grade sodium azide, sodium chloride, sodium hydroxide, sodium phosphate dibasic, citric acid, potassium phosphate monobasic, sodium thiocyanate, and urea were also products of Sigma-Aldrich. Analytical grade potassium chloride, sodium sulfate, hydrogen chloride, sodium hydrogen carbonate, and calcium chloride were bought from BDH Laboratory Suppliers (Poole, UK). Mucin (from porcine stomach, type II) and α-amylase (from porcine pancreas, type VI-B, 22 units/mg solid) were bought from Sigma-Aldrich.

Solution Preparation. WPI and pectin solutions were prepared by adding 1.25 wt % WPI and 0.2, 0.4, 0.8, or 1.6 wt % pectin into phosphate buffers (pH 7), and sodium azide (0.01 wt %) was added to prevent the growth of microorganisms. The solutions were kept overnight to ensure complete dispersion and dissolution. Stock phosphate buffer solutions (pH 3−7), NaCl solutions (0−400 mM, pH 3−7), 0.1 M HCl, and 0.1 M NaOH were also prepared. Deionized water was used to prepare all of these solutions.

Artificial Saliva Preparation. To study the effect of saliva components on volatile release, five artificial salivas with different components were prepared (S1−S5, Table 1).[15] After being stirred for >1 h, salivas were centrifuged at 4000 rpm for 10 min (4 °C) using a Sorvall Legend RT centrifuge (Kendro, Germany) to remove any undissolved substances. The supernatants were collected and stored at 4 °C for future use. The pH of all artificial salivas was adjusted to 6.8 ± 0.2 using 0.1 M HCl or 0.1 M NaOH.

Emulsion Preparation. Primary emulsion was prepared by mixing WPI solution (80 wt % of final emulsion) and sunflower oil at 10000 rpm for 2 min using an ULTRA-TURRAX (IKA, Staufen, Germany) to form a coarse emulsion, which was further homogenized using an M110-EH Microfluidizer with a 75 μm Y-type ceramic interaction chamber (Microfluidics International Corp., Newton, MA, USA) at 50 MPa for three passes. The emulsions were immediately cooled to room temperature (25 °C) using tap water and then stored in an incubator at 25 °C for future use.

For the preparation of multilayer emulsions, pectin solution was added to the primary emulsion (1:1), and the mixture was stirred for 1 h. The pH of the mixture was then adjusted to 5 using 0.1 M HCl. The final multilayer emulsions contained 0.5 wt % WPI, 10 wt % oil, and 0.1−0.8 wt % pectin and were stored at 25 °C for future use.

To study the effects of environmental stresses, primary emulsions and multilayer emulsions were pH adjusted (3−7) using 0.1 M HCl or 0.1 M NaOH and mixed with salt solutions (0−400 mM NaCl, 1:1 dilution) and different artificial salivas (S1−S5, 1:1 dilution, incubated at 37 °C for 5 min before measurements). The subsequent characterization of emulsion properties was finished within 1 h. All of the work was carried out at 25 °C unless otherwise stated.

Emulsion Characterization. Hydrodynamic particle size (z-average) and zeta-potential of emulsions were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. The refractive indices of the particle and water were set at 1.45 and 1.33, respectively. To minimize multiple scattering effects, emulsions were diluted with buffer solutions (same pH and salt concentration as the measured sample) to an oil concentration of ∼0.005 wt % prior to each measurement.[5]

Emulsion stability was evaluated using a multisample analytical centrifuge (Lumifuge, LUM GmbH, Berlin, Germany). The principle of the method was detailed in a previous study.[16] Briefly, emulsion samples were transferred to measurement cells and analyzed by a light beam, which scanned the cells vertically over the total length. The sensor received light transmitted through the sample, which showed a pattern of light flux as a function of the radial position at a given time. On the basis of the evolution of the transmission signal, emulsion instability could be detected. For example, when creaming occurred, the transmission signal at the top of the sample would decrease. In this study, samples were centrifuged at 1500 rpm and 25 °C with a scanning rate of once every 10 s. The result was expressed as the integrated transmission percentage against time, which reflected the creaming stability, with lower values indicating better creaming stability

Flavoring of Emulsions. Volatile solution was prepared by mixing five volatiles in ethanol (5% v/v for each volatile) at room temperature (25 °C) in gastight vials (2 mL, silicone/PTFE seals) (La-pha-pack GmbH, Langerwehe, Germany) and equilibrated by shaking for at least 1 h. Emulsion flavoring was then performed by adding the volatile solution into emulsions in gastight vials (20 mL, silicone/PTFE seals) (La-pha-pack GmbH) to reach a concentration of 500 mg/L for each volatile. The vials were completely filled to minimize volatile losses.

Emulsions were stored at 25 °C before headspace analysis. Preexperiment showed that 1 h of storage was enough for the volatile to reach equilibrium among the different phases of the emulsions.

GC Headspace Analysis. Headspace concentration of volatiles was measured using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA, USA) equipped with a ZB-5MSi capillary column (60 m, 0.25 mm i.d., film thickness = 0.25 μm) and coupled with a FID detector. Flavored emulsion (2 g) was transferred to a 20 mL headspace vial and capped immediately (silicone/PTFE seals) (Lapha-pack GmbH). To study the effects of environmental conditions, the emulsions were rapidly adjusted to the desired pH or mixed with suitable salt solutions or artificial salivas. The vials were incubated at 37 °C for different times (from 30 s to 20 min) in a Combi PAL autosampler (CTC Analytics AG, Switzerland). Pre-experiment showed that sufficient headspace concentration was created after 30 s of incubation, and it was chosen as the start sampling point. Injections of the headspace (1 mL) were performed using a preheated (42 °C) 2.5 mL thermostated gastight syringe (Hamilton, Switzerland) under split mode (1:10). Injector and FID temperatures were, respectively, 225 and 230 °C. The helium carrier gas flow rate was 1 mL/min. The temperature program was as follows: 50 °C (4 min), 200 °C at 10 °C/min rate, 240 °C at 40 °C/min rate (2 min). Results were based on triple analyses.[12]

In this study, initial headspace concentration (sampling after 30 s of incubation; $C_{initial}$) and release rate were adopted to describe volatile release. The kinetics of the volatile release were expressed by plotting the headspace concentration of each volatile against incubation time. Slopes of the initial linear part of the release curves were taken as release rates (mg/L min).

Statistical Analysis. Statistical analysis was performed using OriginPro 7.5. Measurements were repeated at least three times. A one-way analysis of variance (ANOVA), followed by Tukey's test, was applied to determine significant differences between the mean values of each test. A significance level of $p < 0.05$ was used throughout the study.

RESULTS AND DISCUSSION

Formation and Properties of Multilayer Emulsion. *Effect of pH.* pH plays an essential role in the formation of multilayer emulsions.[4] Zeta-potential analysis showed that droplets in primary emulsions were negatively charged at pH 7 and 6 and positively charged at pH 4 and 3 (Figure 1A). Pectin carries only negative ions regardless of the pH of the solution. With the addition of pectin, emulsion droplets could have different charge signs and magnitudes from those in the primary emulsion. Specifically, at pH 5, 4, and 3, droplets carried more negative charge in emulsions with higher pectin content, and the magnitude of the charge decreased with decreasing pH. In emulsions with 0.4 and 0.8 wt % pectin, the droplets were negatively charged throughout the pH range studied (pH 7 to 3). The results indicate that a pectin layer was formed at the droplet surface at pH 5, 4, and 3. The formation of the pectin layer was driven by electrostatic forces, as WPI and pectin were thought to be oppositely charged at pH below the isoelectric point (p*I*) of WPI. When the pH of the emulsions was lowered from 5 to 3, the protein layer had higher positive charge and more pectin was adsorbed. However, at neutral pH 7 and 6, addition of pectin to primary emulsion did not present a significant effect on the charge intensity, as pectin and WPI were both negatively charged. It is worth pointing out that although WPI–pectin interaction could also occur at neutral pH through hydrogen bonding, hydrophobic, and/or electrostatic attraction, etc., these forces were relatively weak.[17]

The droplet size of the emulsions was influenced by both pectin content and pH (Table 2). For some emulsions with lower pectin contents, severe droplet aggregation occurred at

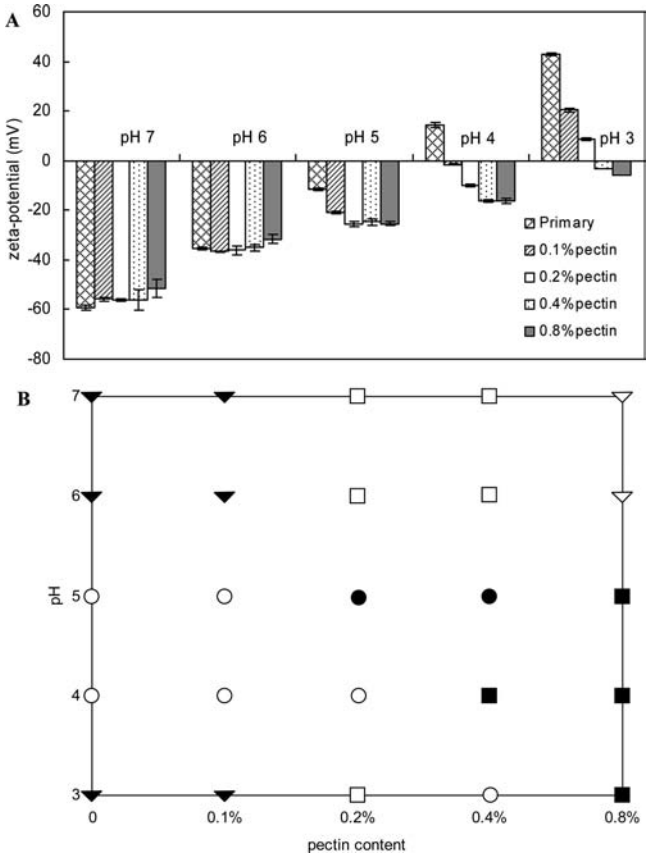

Figure 1. Effect of pH on the properties of emulsions (0.5 wt % WPI, 10 wt % oil) with different pectin contents: (A) zeta-potential; (B) stability map based on Lumifuge test; (solid symbols) emulsions unseparated after stability test (level of droplet aggregation: ■ < ● < ▼); (open symbols) emulsions separated after stability test (creaming rate: ▽ < ○ < □).

pH 5 and 4, as the droplets were poorly charged (WPI has an isoelectric point of 4 < pH < 6). Meanwhile, emulsions differing in pectin content allowed contrasting stability determined by Lumifuge (Figure 1B). Primary emulsions were unstable at pH 5 and 4, and phase separation induced by rapid droplet aggregation was observed soon after the start of the stability test. Addition of 0.1 wt % pectin into the emulsion showed no improvement in emulsion stability. When pectin content was >0.2 wt %, the emulsions showed better stability against lower pH. For example, at 0.8 wt % pectin multilayer emulsions were very stable at pH 5, 4, and 3, and no creaming was detected. Steric hindrance between droplets was mainly responsible for the stability of multilayer emulsions.[4] Adsorption of protein–polysaccharide complex at interfaces could increase interfacial viscosity, creating a gel-like structure surrounding the oil droplets and preventing droplet aggregation.[18] The stability map also showed that emulsions were unstable at neutral pH with the addition of pectin (≥0.2 wt %), which was induced by depletion flocculation as most pectin was present in the continuous phase.[4] It should be noted that the stability test was conducted within a centrifuge field, and the destabilization process was well accelerated. In fact, multilayer emulsions at neutral pH did not show creaming in the initial 48 h. The multilayer emulsions formed at pH 5 were chosen for the rest of the study (response to environmental conditions) to expand the use of WPI at pH close to its p*I* value.

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Table 2. Effect of pH on the Hydrodynamic Particle Size (Nanometers) of Emulsions (0.5 wt % WPI, 10 wt % Oil) with Different Pectin Contents (Mean ± SD, $n \geq 3$)

	pectin content				
pH	0 wt %	0.1 wt %	0.2 wt %	0.4 wt %	0.8 wt %
7	205.8 ± 2.5	197.1 ± 1.9	204.3 ± 4.5	202.9 ± 5.6	257.0 ± 9.9
6	216.7 ± 0.5	228.3 ± 4.8	238.6 ± 6.9	230.1 ± 3.7	318.2 ± 10.6
5	–[a]	–	1068.3 ± 25.1	630.7 ± 14.9	440.6 ± 8.4
4	–	–	–	348.2 ± 5.6	374.8 ± 15.7
3	230.2 ± 3.0	730.3 ± 51.3	–	–	394.0 ± 13.0

[a]Particle size was not reported as severe droplet aggregation formed and the size was beyond the measurement limit.

Effect of Salt. Primary emulsions and multilayer emulsions were subjected to salt solutions with a range of NaCl concentrations, and emulsion properties were greatly modified (Figure 2). Interfacial charge (absolute value) of the emulsions decreased with the increase of salt content (primary emulsion and multilayer emulsion with 0.1% pectin were at pH 3, and the other emulsions were at pH 5, as the two former emulsions were unstable at pH 5), which was caused by the "electrostatic screening" effect. Adsorption of either Na^+ or Cl^- onto the interface neutralized some of the ions from pectin or WPI, resulting in reduced interfacial charge.[19] Consequently, severe droplet aggregation occurred at salt concentration >100 mM (data not shown). Stability tests showed that when multilayer emulsions were subjected to 150 or 200 mM NaCl solutions, optical transmission drastically increased to the peak level in the first 10 min and remained almost unchanged thereafter, suggesting rapid phase separation at the beginning of centrifuging. In emulsions subjected to 100, 50, or 0 mM NaCl solutions, a slight increase of transmission signal was observed after 4 h test, and no phase separation occurred. The mechanism of salt-triggered emulsion instability is well understood.[19] The screening effect reduces the repulsion force between droplets, and the resulting force is not sufficient to overcome the attraction forces (e.g., van der Waals, hydrophobic force), leading to droplet association. Second, salt ions could decrease the thickness and increase the porosity of WPI–pectin double layer by weakening the interaction between the two layers.[20] It has been reported that in protein-stabilized emulsions the surface concentration of protein may increase because salt can reduce the repulsion force between adsorbed and unadsorbed protein.[20] In a multilayer emulsion, this phenomenon may be hindered by the presence of the pectin layer. Some studies reported that multilayer emulsions had better stability against higher salt concentration (or ion strength) than primary emulsions,[6,8] whereas in the current study primary emulsions had better stability. The contradiction could be due to the different pH chosen for the stability test.

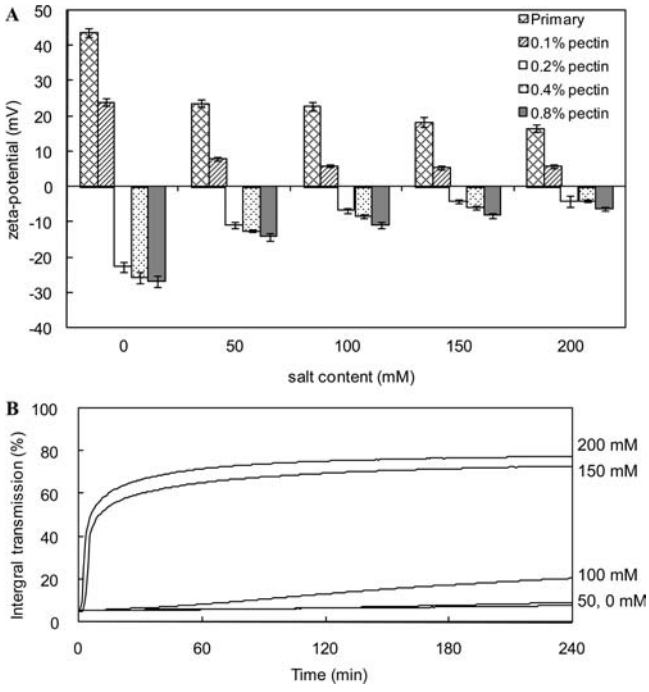

Figure 2. Effect of salt content (0–200 mM) on the properties of emulsions (0.5 wt % WPI, 10 wt % oil) with different pectin contents: (A) zeta-potential (primary emulsion and multilayer emulsion with 0.1 wt % pectin were at pH 3; multilayer emulsions with pectin content from 0.2 to 0.8 wt % were at pH 5); (B) emulsion stability (pectin content 0.5 wt %, pH 5). The slope of the integral transmission–time curve is an indicator of creaming stability; the higher the slope, the lower the stability was.

Effect of Artificial Salivas. Liquid foods, for example, emulsions, normally stay in the mouth for only several seconds before swallowing. During their residence, emulsion properties could be influenced by salivas. The influences include saliva dilution, heating or cooling, and interactions between saliva components (salts, enzymes, biopolymers, etc.) and emulsion components.[21−23] When multilayer emulsions were subjected to different artificial salivas, significant changes in interfacial charge of the droplets were first observed (Figure 3A). Droplets in emulsions diluted with salivas (1:1) containing salt, mucin, and/or α-amylase (S2–S5) had about 50% intensity of the zeta-potential of the droplets in undiluted emulsions. The lowest magnitude of surface charge was found in S2 (salt alone) treated emulsion. Dilution with S1 (water alone) did not change the zeta-potential significantly ($p > 0.05$), and the pH of the emulsion remained unchanged (data not shown). Emulsions diluted with other salivas (S2–S5) had significantly higher pH (~7) than the original multilayer emulsion (pH 5). This suggested that salt, mucin, and/or α-amylase in S2–S5 were the main factors influencing droplet charge. As stated earlier, salt produced a screening effect and reduced the magnitude of interfacial charge. pH neutralization led to partial detachment of pectin from the interface, resulting in even lower charge density. A small quantity of the proteins (α-amylase and mucin) in the salivas could be attracted by positively charged patches of the interfacial layer,[23] which was responsible for the higher magnitude of the zeta-potential of S3–S5 diluted emulsions.

Figure 3B shows that S5 diluted emulsion was the least stable, followed by S3, S4, and S2 diluted emulsions. The instability of these emulsions originated from droplet

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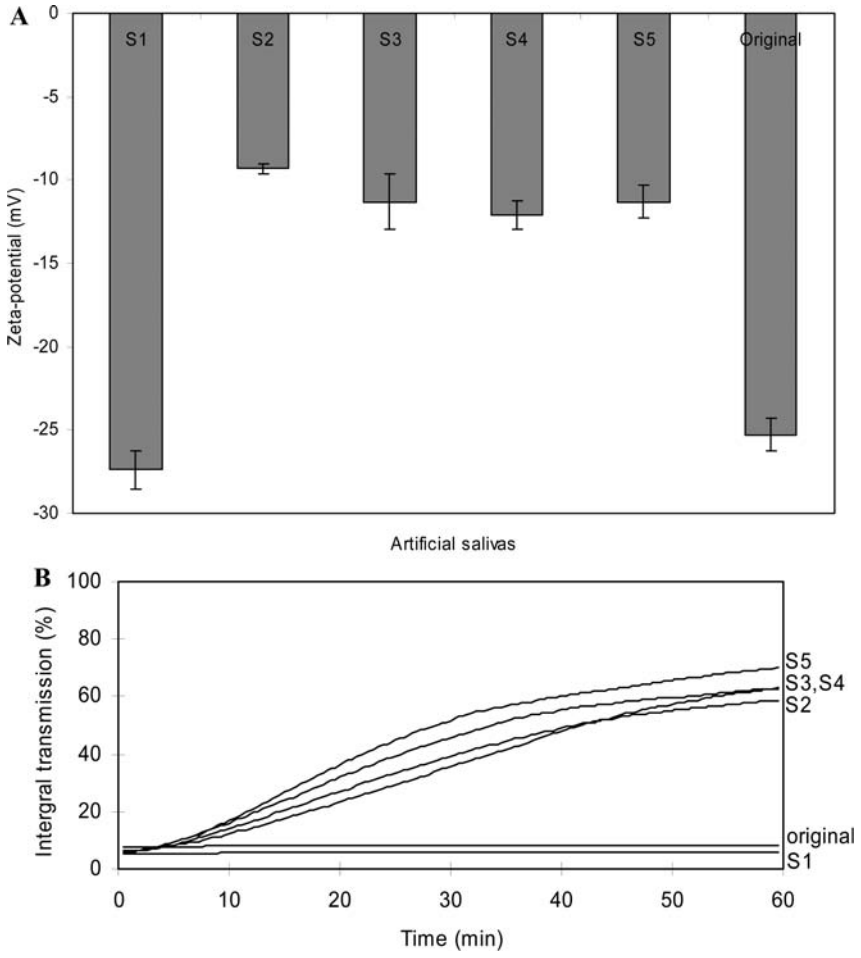

Figure 3. Effect of different artificial salivas on the properties of multilayer emulsions (0.5 wt % WPI, 10 wt % oil, 0.8 wt % pectin, pH 5): (A) zeta-potential; (B) emulsion stability. The slope of the integral transmission−time curve is an indicator of creaming stability; the higher the slope, the lower the stability was. The composition of each saliva is presented in Table 1.

flocculation.[24] Pre-experiment microscopic observation found that undiluted multilayer emulsion had small droplets with fine distribution. In saliva diluted systems, some droplets aggregated and many bigger droplets were observed.

Volatile Release from Multilayer Emulsion. In response to environmental conditions, release behavior of volatiles could be well modified. The modification was closely linked to emulsion properties. In this part of the study, the release behavior of 1-propanol, diacetyl (butane-2,3-dione), 2-pentanone, ethyl butyrate, and 2-heptanone was tested. The selection of these compounds was based on their physicochemical properties, including the chain length, function group, volatility, polarity, etc. Initial headspace concentration ($C_{initial}$) and release rate provide information about the temporal release of volatile compounds and were used to describe volatile release from emulsions under different pH, salt concentrations, and artificial salivas.

Effect of Pectin Content. $C_{initial}$ and release rates of the volatiles from primary emulsion and multilayer emulsions are presented in Figure 4. Volatiles in the multilayer emulsions had lower $C_{initial}$ than those in the primary emulsion, more significantly for ethyl butyrate and heptanone (Figure 4A). With the increase of pectin content from 0.4 to 0.8 wt %, a higher decrease of headspace concentration occurred. Multilayer emulsion has an interfacial film with higher thickness and stronger mechanical properties, which could retard mass transfer of volatile molecules across the oil−water interface. Second, higher viscosity of multilayer emulsions slowed volatile diffusion between different phases.[25,26] In pectin−water systems, volatiles could also be trapped in the pectin gel network.[27] Third, both pectin and protein (adsorbed or unadsorbed at the interface) were able to adsorb volatiles. WPI (or β-lactoglobulin) could bind ketones,[28,29] esters,[30] or aldehydes[31] through hydrophobic interaction or covalent binding.[32−34] The former occurred in the hydrophobic pocket (the central calyx) of the protein,[33] and the latter could happen at protein surfaces.[35] In pectin-containing systems, many volatiles had lower headspace concentration. The interaction between volatile compounds and pectin could proceed through van der Waals interaction between the alkyl patch of a volatile molecule and the hydrophobic region of pectin.[36] Besides, hydrogen atoms in the undissociated carboxyl group of pectin could interact with unshared electron pairs of heteroatoms and oxygen atoms of volatile molecules via hydrogen bonding.[37] Due to the unfolded conformation of protein and the competitive binding of volatiles by protein and pectin, protein at the multilayered interface could attract fewer volatile compounds than the protein at the single-layered interface.[38] The different $C_{initial}$ values of volatiles in the two multilayer emulsions were mostly due to the difference in pectin content, mainly the unadsorbed part. The droplets in the two multilayer emulsions possibly had the same amount of pectin covered, as

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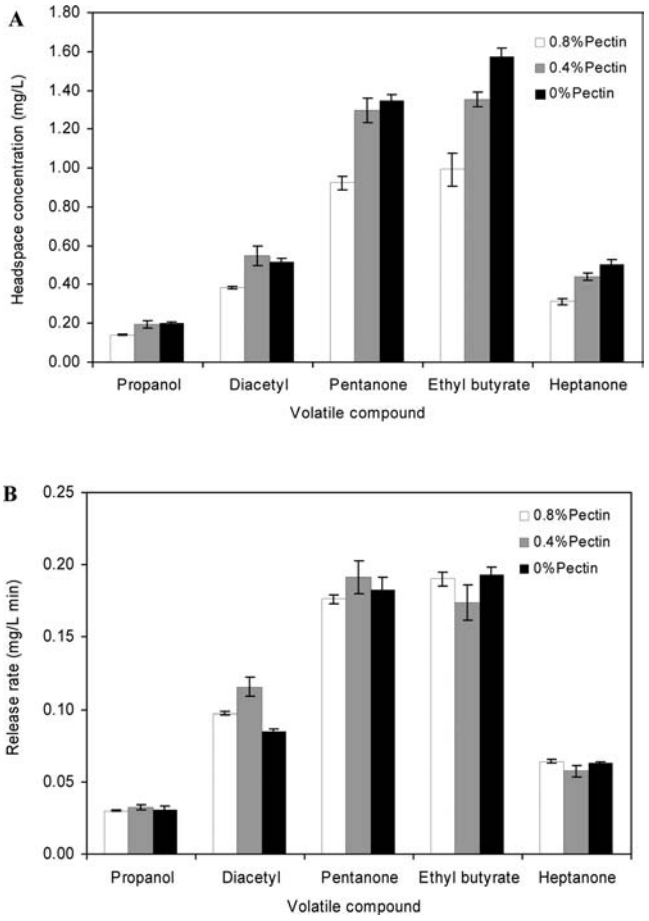

Figure 4. Volatile release from emulsions with different pectin contents (0.5 wt % WPI, 10 wt % oil, pH 5): (A) initial headspace concentration (sampling after 30 s of incubation at 37 °C); (B) release rate (samples were incubated at 37 °C before each measuring point).

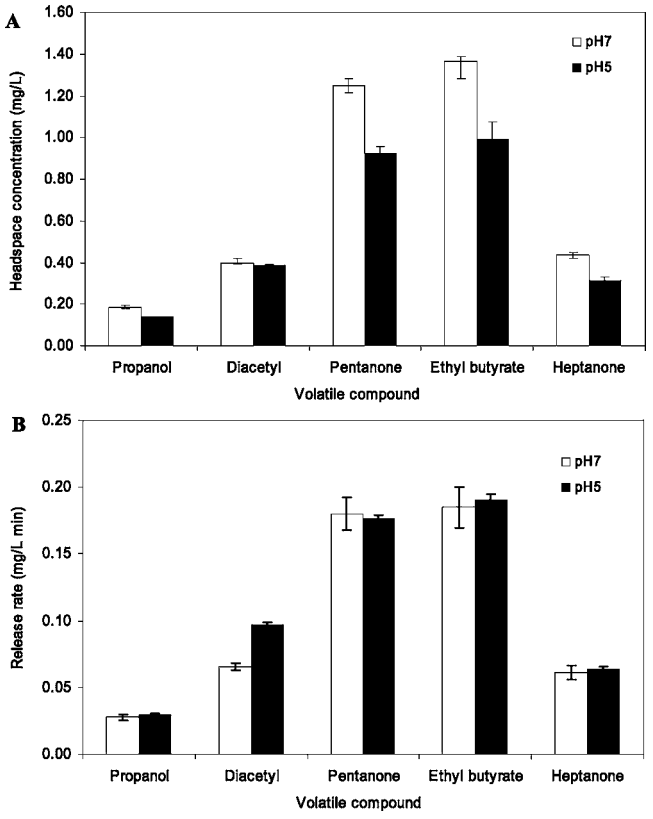

Figure 5. Effect of pH adjustment on the release of volatile compounds from multilayer emulsions (0.5 wt % WPI, 10 wt % oil, 0.8 wt % pectin); (A) initial headspace concentration (sampling after 30 s of incubation at 37 °C); (B) release rate (samples were incubated at 37 °C before each measuring point).

they had significantly the same zeta-potential. However, the pectin layers may spread differently (due to different interfacial areas) and thus differ in compactness and porosity.

Interestingly, addition of pectin did not produce a significant effect on the release rate of most volatile compounds (Figure 4B). In Benjamin et al.'s study,[12] hydrophobic compounds were releasing at lower rates in primary emulsion, and the author attributed the result to the hindering diffusion effect of large particles in the monolayer emulsion. This hypothesis could be applicable to the current system, as the droplet size of the primary emulsion (droplet aggregates) was several times bigger than that of the multilayer emulsion (Table 2). Due to the shortened transportation radius of the submicrometer particle, volatiles were moving faster in multilayer emulsions,[39] whose role counteracted the barrier effect of the multilayer.

Effect of pH. When multilayer emulsions were pH adjusted (to pH 7), different release behaviors of the volatiles were observed (Figure 5). All of the volatiles had significantly increased $C_{initial}$ after pH adjustment, more prominently for lipophilic volatiles, that is, pentanone, ethyl butyrate, and heptanone (Figure 5A). At pH 7, both pectin and WPI were negatively charged,[4] and the interaction between these two ingredients was weakened. A considerable amount of pectin could detach from the interface, and the remaining pectin could pack in a loose style. Modification of pH could also influence the porosity of the multilayer at the interface of nanoparticles.[40] Therefore, volatile molecules would move more freely across the interface at pH 7. As lipophilic volatiles were largely distributed inside the oil droplets, their release behavior was great affected due to modification of the interfacial properties. In comparison with volatile release from primary emulsions (pH 7) (data not shown), volatile release from pH-adjusted multilayer emulsion was at a significantly lower level, which could be due to the interaction between volatile compounds and pectin (adsorbed or unadsorbed). Moreover, change of pH may alter the tertiary structure of protein and the pK value of each volatile compound, which could also modify the release behavior of volatiles.[41,42] In this study, change of pH did not show a significant effect on the release rates of volatiles, with the exception of diacetyl (Figure 5B), which was released at lower rates in emulsions at pH 7. A similar result for diacetyl was reported in egg yolk or starch sodium octenylsuccinate stabilized emulsions, in which increase of pH from 3 to 9 resulted in an enhanced retention of diacetyl.[43] The result was attributed to the strengthened interaction between diacetyl and the stabilizers through electrostatic attraction or hydrogen bonding at higher pH conditions.

Effect of Salt. Although salt plays an important role in flavor perception, the effect of salt on volatile release from emulsions has seldom been reported.[12,13] The presence of 100 mM NaCl in the emulsions led to significant increases in $C_{initial}$ of all the volatiles. A further increase of NaCl concentration from 100 to 200 mM did not change $C_{initial}$ significantly ($p > 0.05$). Meanwhile, all of the volatiles had higher release rates in emulsions diluted with salt solutions than in undiluted

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emulsions (Figure 6). The phenomenon that addition of salt can increase flavor release is called "salting-out". It is due to the reduction in the number of water molecules available to solubilize flavor compounds.[44] However, in multilayer emulsions salt ions were mostly involved in the screening effect and less involved in the salting-out effect.[12] WPI–pectin interaction at the interface was weakened due to the screening effect, and the interface cannot well retard volatile movement and can adsorb fewer volatile compounds. During the short period of a GC test the salt did not induce phase separation, and 100 or 200 mM NaCl had the same effect on volatile release. A similar result was reported in a β-lactoglobulin–pectin stabilized emulsion, in which increase of salt content from 100 to 500 mM did not significantly influence volatile release.[12] It is worth pointing out that the multilayer would collapse after a longer time of storage under a higher concentration of NaCl, fewer volatiles would be retained by the emulsion, and they would have higher release rates.

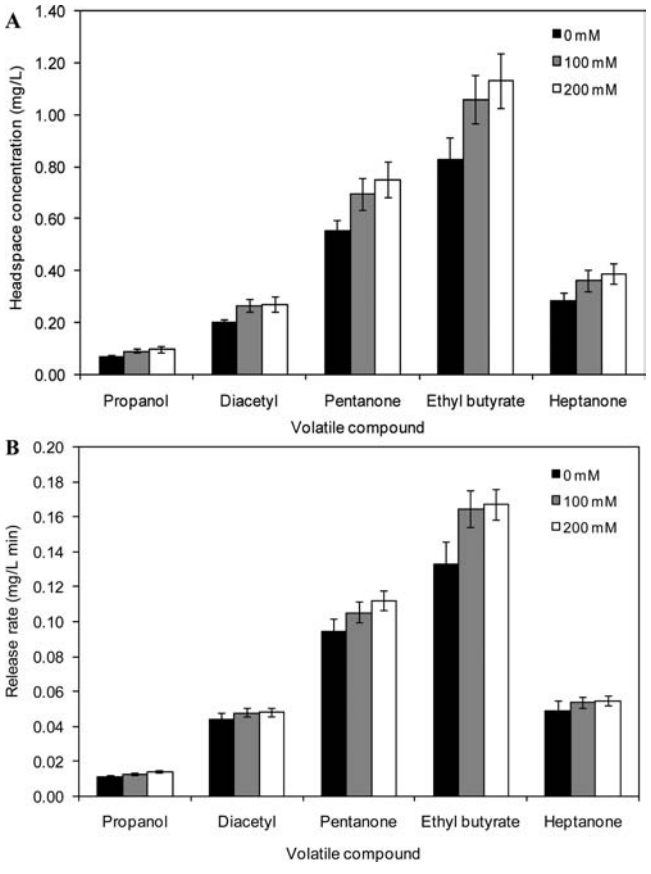

Figure 6. Effect of NaCl concentration on the release of volatile compounds from multilayer emulsions (0.5 wt % WPI, 10 wt % oil, 0.8 wt % pectin, pH 5), 1:1 dilution with NaCl solution; (A) initial headspace concentration (sampling after 30 s of incubation at 37 °C); (B) release rate (samples were incubated at 37 °C before each measuring point).

Effect of Artificial Salivas. In the oral cavity, volatile release from emulsions is largely influenced by saliva.[45] Apparently, dilution with saliva will first disturb the partition and mass transfer of volatiles in the aqueous and oil phases, leading to different release kinetics. In the current study, three representative artificial salivas (S3, S4, and S5) were tested, and the dilution effect was more prominent for volatiles with higher hydrophilicity (Figure 7). For example, in untreated multilayer emulsion diacetyl had a $C_{initial}$ of 0.384 mg/L and a release rate of 0.097 mg/L min (Figure 5, pH 5), whereas in S5 diluted emulsion it had a $C_{initial}$ of 0.178 mg/L and a release rate of 0.029 mg/L min (Figure 7). Although salts were present in each artificial saliva, the salting-out effect could be masked by the dilution effect.[46] Compared to buffer-treated emulsions, saliva-treated emulsions generated higher release of hydrophobic compounds (e.g., pentanone, ethyl butyrate, and heptanone) and lower release of hydrophilic compounds (e.g., diacetyl). A similar result was reported in a flavored pectin gel system and could be attributed to the increased hydrophilic properties of the system when diluted with artificial salivas.[47] The proteins (mucin, α-amylase) in the salivas were likely to bind larger, more hydrophobic compounds, which then reduce the headspace concentration of these volatiles.[48] However, this trend was not observed in the current study, possibly because the interactions were rather weak or the trend was masked by rapid volatile release triggered by emulsion instability. Emulsion diluted with S5 underwent the highest droplet flocculation and most rapid phase separation, which could account for the highest release of volatiles from S5 diluted emulsion. It should be noted that in the oral cavity, air

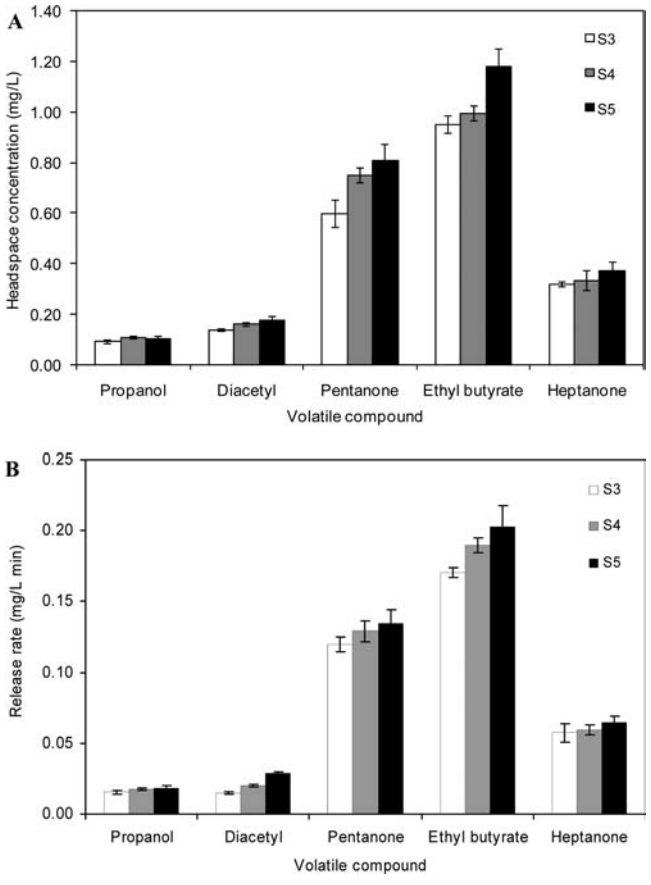

Figure 7. Effect of artificial salivas on the release of volatile compounds from multilayer emulsions (0.5 wt % WPI, 10 wt % oil, 0.8 wt % pectin, pH 5), 1:1 dilution with saliva: (A) initial headspace concentration (sampling after 30 s of incubation at 37 °C); (B) release rate (samples were incubated at 37 °C before each measuring point). S3 contained a mixture of salts and mucin. S4 contained a mixture of salts and α-amylase. S5 contained a mixture of salts, mucin, and α-amylase. Detailed composition of each saliva can be found in Table 1.

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flow, temperature, tongue movement, and other factors can also affect volatile release.[49] A more complicated mouth model or in vivo study could be considered for future study to better understand oral release behavior.

This work presented the application of WPI−pectin multilayer stabilized emulsions as delivery systems for volatile compounds under different environmental conditions. The results demonstrate that multilayer emulsions can reduce the amount of volatiles released to the headspace, especially the initial release. The ability of multilayer emulsions to mediate volatile release was highly affected by emulsion properties. When a compact second layer was formed over the preadsorbed WPI layer, the interface could well retard volatile movement and adsorb more volatile compounds, thereby reducing the amount of volatiles released to the headspace. Under certain conditions, such as neutral pH, a high concentration of salt, or salivas, the interaction between the two layers could be weakened. It then resulted in thinner interfacial film and detachment of pectin, and volatile release could proceed more freely. This provides an option to get the desired volatile profile of certain foods by interfacial engineering under controlled environmental conditions.

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Notes

The authors declare no competing financial interest.

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