The use of spray drying to microencapsulate 2-acetyl-1-pyrroline, a major flavour component of aromatic rice

Muanmai Apintanapong & Athapol Noomhorm*

Processing Technology Program, Post-Harvest and Food Process Engineering, School of Environmental, Resources and Development, Asian Institute of Technology, PO Box 4, Klongluang, Pathumthani 12120, Thailand

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Summary

Different ratios of gum acacia and maltodextrins were used to investigate the appropriate wall materials for encapsulation, by spray drying, of 2-acetyl-1-pyrroline (ACPY). This compound, which is the major flavour component of aromatic rice, was extracted from pandan (Pandanus amaryllifolius) leaves by steam distillation. The amounts of ACPY before and after spray drying encapsulation were not significantly different ($P < 0.05$). Better retention of ACPY was obtained by encapsulation. In its liquid form ACPY degraded quickly as 63% reduction occurred in a basic solution after 7 days; however, only 30% reduction was found in acidic solution after 35 days of storage. After 72 days of storage, the amount of ACPY in encapsulated powders made with differing amounts of gum acacia and maltodextrin decayed as follows: ratios of gum acacia:maltodextrin of 70:30, 60:40, 50:50, 40:60, 30:70 and 0:100 gave the following amounts of degradation of 27.7, 33.4, 43.2, 35.7, 30.6 and 32.6% respectively. Encapsulation in 70:30 gum acacia:maltodextrin gave the best retention of ACPY.

Keywords Aroma, gum acacia, maltodextrin, Pandanus amaryllifolius, steam distillation extraction.

Introduction

The compound 2-acetyl-1-pyrroline (ACPY) was identified as an important compound contributing to popcorn-like aroma in several Asian aromatic rice varieties (Buttery et al., 1982). This characteristic sweet aroma, found in Basmati rice, is released when the grain is cooked (Bhattacharjee et al., 2002). It was positively correlated with descriptive terms such as popcorn-like aroma by non-orientals and pandan-like aroma by orientals (Paule & Powers, 1989). ACPY is chiefly responsible for the characteristic odour of aromatic rice varieties according to Lin et al. (1989) and Tanchotikul & Hsieh (1991). ACPY was also isolated and identified from other sources such as pandan leaves (Buttery et al., 1982, 1983; Laksanalamai & Illangamileke, 1993), the crust of wheat and rye breads (Schieberle & Grosch, 1987; Schieberle, 1990), popcorn (Schieberle, 1991), and some sweet corn products (Buttery et al., 1994).

The use of ACPY and its salts as flavouring ingredients, particularly in imparting an ‘aromatic rice flavour’ to foods or in flavour modifying compositions, are now patented (Buttery et al., 1984, 1985). The compound is synthesized chemically and can be added to nonaromatic rice varieties and rice-containing dishes including many kinds of pilaf, pudding and the like. It can also be used to flavour meat and vegetable products or blended with other flavour enhancing sauces or compositions. ACPY may be added directly to the food or flavouring agent in very small quantities because of its very low threshold (0.1 ppb). Stable solid salts of ACPY can be prepared with physiologically acceptable acids such as hydrochloric acid and citric acid (Buttery et al., 1984, 1985).

Those markets importing aromatic rice are reported to have expanded in recent years in order
to meet the individual preferences of consumers (Laksanalamai, 1993). However, there is a problem in the storage of the aromatic rice as ACPY, the major aromatic component, seems to decrease with storage time (Laksanalamai & Ilangantileke, 1993). One method of improving the quality of odour in products is to add ACPY compound to the rice. The powdered form of ACPY may be produced by encapsulation and applied to the rice or other foods according to the amount of its potent, pleasing characteristic flavour required. In the flavour industry, encapsulation is the most popular modern technique of converting a volatile aroma concentrate to a stable powder form (Sankarikutty et al., 1988; Reineccius, 1991).

Encapsulation can be defined as the technique of packing minute particles of a core material within a continuous polymer film. This method is designed to release its contents in a predictable manner under a predetermined set of conditions (Sankarikutty et al., 1988). A number of methods have been reported for the microencapsulation of flavours, but the most popular technique employed in the industry is spray drying (Sankarikutty et al., 1988). It is still the most economical and widely used method of encapsulation, finding broad use in the flavour industry (Risch, 1995).

In selecting the wall materials for encapsulation, maltodextrin is a good compromise between cost and effectiveness, as it is bland in flavour, has low viscosity at a high solid ratios and is available in different average molecular weights. Maltodextrin, with increasing dextrose equivalent value, offers increased protection to encapsulated orange oil, most possibly by virtue of its improved oxygen-barrier properties (Anandaraman & Reineccius, 1986). The encapsulation of pure β-carotene in twenty-five dextrose equivalent maltodextrin by spray, freeze and drum drying was investigated by Desobry et al. (1997). Another wall material is gum acacia, the traditional carbohydrate of choice for encapsulation via spray drying. An interesting and unique property of gum acacia is its low viscosity in aqueous solution (Reineccius, 1991). In several cases, mixtures of these compounds can be used to obtain better results. Moreover these two ingredients, maltodextrin and gum acacia, are edible and approved by the Food and Drug Administration. Mixtures of gum acacia and maltodextrin showed promise as high solid carriers, giving acceptable viscosity in studies on microencapsulation of cardamom oil by spray drying (Sankarikutty et al., 1988).

To test the hypothesis that gum acacia and maltodextrin would protect the flavour of ACPY from undesirable interactions, they were selected as wall materials for ACPY encapsulation by spray drying. The appropriate ratio of wall material, gum acacia and maltodextrin, for ACPY encapsulation was investigated. Loss of ACPY during storage was also determined in this study.

Materials and methods

Chemicals

Collidine (2,4,6-trimethylpyridine, TMP) was purchased from Sigma Co., Ltd (St Louis, MO, USA) and used as an internal standard in gas chromatographic analysis. The stock solution of collidine of 125 ppm concentration in water was prepared and stored at 4°C. Gum acacia and maltodextrin (15 D.E.), used in encapsulation, were purchased from Sigma Co., Ltd and Theppratan Development Co., Ltd. (Bangkok, Thailand), respectively.

Preparation of ACPY

The ACPY was isolated from pandan leaves (Pandanus amaryllifolius) by steam distillation extraction (SDE) originally described by Likens & Nickerson (1966). The extraction method was adopted from Rungsardthong (1995). Fresh pandan leaves (400 g) were blended and mixed with 1.5 L of volatile-free distilled water, obtained by boiling 4.25 L of distilled water in an 8-L round-bottom flask. When the volume of water in the boiling flask was reduced to about 4 L, the filtrate of the pandan mixture was then added, and the boiling flask with the filtrate of pandan mixture was placed in the steam distillation extraction apparatus. Distilled water was added to fill the U-tube located beneath the column of the distillation extraction apparatus.

The SDE column was cooled using a circulating water-cooling system, then the right arm of the SDE apparatus was attached to the neck of the
12-L flask. The left arm of the apparatus was attached to a 250-mL round-bottom solvent flask containing 80 mL of distilled water, 2 mL of diluted sulphuric acid and 120 mL of diethyl ether. The 250 mL solvent flask was placed in a 500-mL beaker containing 100 mL of water. This served as a water-bath to heat the diluted sulphuric acid–diethyl solvent mixture. Water temperature in the 500 mL beaker was controlled by a 50 °C setting on a hot plate, which was adequate to evaporate the diethyl ether at a boiling point of 39.5 °C.

When the pandan leaves sample in the 12-L flask began to boil, the hot plate temperature was lowered to prevent vigorous boiling. The solvent mixture was refluxed and stirred vigorously. After 2 h of continuous distillation extraction, the solvent flask was removed, and the sulphuric acid layer was separated into a 250-mL Erlenmeyer flask, using a 250-mL separating funnel. The acid solution was collected and evaporated in a rotary evaporator to remove all ether. This water layer was kept in the refrigerator and used for acidic and basic ACPY solutions and ACPY encapsulation.

Gas chromatographic-mass spectrometric (GC-MS) analysis

An aliquot (0.5 μL) of the extracted sample was injected into a Varian series 3400 cx gas chromatograph (Varian Medical Systems, Inc., Palo Alto, CA, USA) with a flame ionization detector to determine the presence of ACPY. The instrument and condition specifications were as follows: a 30-m capillary column DB-5 MS (5% diphenyl–95% dimethyl polysiloxane; J&W Scientific, Agilent Technologies Inc., Palo Alto, CA) with an inside diameter of 0.25 mm was used with helium carrier gas at a flow rate of 1 mL min⁻¹ and a split rate of 90 : 1, injector and detector temperatures were 220 °C and the capillary column was maintained at 50 °C for 1 min, the temperature of which gradually increased at a rate of 10 °C min⁻¹ to 220 °C and was then kept constant.

A Saturn 4D mass selective detector (Varian Medical Systems, Inc., Palo Alto, CA, USA) in electron impact (EI) ionization mode was used to confirm the presence of ACPY in the sample. Mass-spectra were obtained by focusing on the peak eluting at about 5.33 and 6.45 min.

Preparation of acidic and basic ACPY solutions

Acidic ACPY solution

The ACPY extracted from pandan leaves (P. amaryllifolius) by the SDE method was collected and the ether removed by evaporation, resulting in a solution with a pH of about 2.

Basic ACPY solution

The basic ACPY solution was prepared by adding sodium bicarbonate to the acidic ACPY solution until pH 8 was reached.

Stability of ACPY in liquid form

Acidic ACPY solution

10 mL of acidic ACPY solution (approximately 30 ppm ACPY) was placed in a 16 × 150 mm test tube with a screwed cap and stored at ambient temperature for 35 days. The ACPY concentration was determined at 0, 9, 20 and 35 days by GC analysis.

Basic ACPY solution

10 mL of basic ACPY solution (approximately 30 ppm ACPY) was placed in a 16 × 150 mm test tube with a screwed cap and stored at ambient temperature for 7 days. The ACPY concentration was determined at 0, 1, 2, 3 and 7 days by GC analysis.

Wall material characterization

Different ratios of gum acacia and maltodextrin (DE 15) (as shown in Table 1) were hydrated prior to use.

<table>
<thead>
<tr>
<th>Gum acacia: maltodextrin</th>
<th>Weight of wall materials† (gram dry weight 100 mL⁻¹ solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 : 0*</td>
<td>26</td>
</tr>
<tr>
<td>70 : 30</td>
<td>33</td>
</tr>
<tr>
<td>60 : 40</td>
<td>33</td>
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<tr>
<td>50 : 50</td>
<td>33</td>
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<tr>
<td>40 : 60</td>
<td>33</td>
</tr>
<tr>
<td>30 : 70</td>
<td>33</td>
</tr>
<tr>
<td>0 : 100</td>
<td>36</td>
</tr>
</tbody>
</table>

*Not pumpable.
†Source: Sankarikutty et al. (1988).
Note: Moisture contents of gum acacia and maltodextrin were 88.76 and 93.95%, respectively.
to spray drying. The gum solutions were prepared in warm (50–60 °C) water to aid dispersion. The ACPY stock solution (approximately 40 ppm ACPY) was added and mixed with the wall material solution to prepare a sample mixture with approximately 10 ppm of ACPY.

**Encapsulation of ACPY**

The mixture was spray-dried rapidly, by using a counter-current method, in a laboratory scale spray dryer (model SD-05; Laboratory-Plant Limited, Huddersfield, UK), equipped with a nozzle of 0.5 mm diameter and inside chamber dimensions of 50 cm height and 21.5 cm outside diameter. Drying conditions were standardized at an inlet temperature of 150 ± 5 °C and exit air at 80 ± 5 °C. Pump flow rate was set at 475 mL h⁻¹ with an air flow rate of 63 m³ h⁻¹ (to maintain the exit air temperature at 80 °C).

**Determination of moisture content**

Moisture content was determined using the oven drying method (AOAC, 1984).

**Storage and ACPY analysis**

Encapsulated powders from spray drying were stored in closed glass containers at ambient temperature. The change in ACPY during storage was monitored by GC. The storage period for the spray drying was 72 days and analysis of ACPY content was done after 0, 10, 20, 30, 50 and 72 days.

A Shimadzu series GC-14B gas chromatograph (Shimadzu Corporation, Kyoto, Japan) and a 2.5 m x 3 mm i.d. pyrex glass packed column (Supelco, Sigma-Aldrich Inc., St. Louis, MO, 20% Silicone DC-550 on 60/80 mesh Chromosorb WHP) were used for the analysis of ACPY, using collidine as an internal standard (Buttery et al., 1986). The GC detector was of the flame ionization type and nitrogen gas was used as carrier gas (30 mL min⁻¹). The analysis was done using a constant column temperature of 110 °C and the temperature of the injector and detector was set at 170 °C. The volume of the injected sample was 2 μL.

The ACPY extraction method of Rungsardthong (1995) was used. A 5 g sample was dissolved in 10 mL of distilled water and the pH adjusted to 8.0 with sodium bicarbonate. 150 μL of collidine solution and 4 mL of diethyl ether were added to the mixture and shaken using a Vortex mixer (Genic 2 model G360E, Scientific Industries Inc., NY). The amount of ACPY and collidine in the ether layer were determined by GC analysis.

The concentration of ACPY in the sample was calculated using the equation:

\[
\text{ACPY concentration (mg L}^{-1}\text{)} = \frac{\text{Area of ACPY peak}}{\text{Area of collidine peak}} \times \frac{\text{added collidine (μg)}}{10\text{mL}} \times \text{RRF}
\]

where RRF is the per cent of collidine and ACPY recovered from the extract.

**Data analysis**

The amounts of ACPY during storage were statistically analysed. The analysis of variance (ANOVA) and comparison of mean values were determined using Statgraphics (Statistical Graphics Corporation, Englewood Cliffs, NJ, USA). Significant differences between treatment mean values were determined using the Duncan multiple range test.

**Results and discussion**

Figure 1 shows the separation of ACPY in GC using a DB-5 MS capillary column. The mass spectrum of the peak at 5.33 min is shown in Fig. 2. The mass spectrum obtained [with a molecular ion at mass/charge (relative ion intensity) 111 (5) and other major ions at 43 (100), 41 (50), 42 (24), 83 (11), 69 (11), 68 (8), 55 (2), 52 (0.9), 54 (0.2), 67 (0.2)] was similar to that of the authentic ACPY reported by Buttery et al. (1983). Therefore, it is possible to use ACPY solution extracted from pandan leaves as the core mixture for ACPY encapsulation.

In the experiment, the mixture obtained with 100% gum acacia was not pumpable because of high viscosity. After spray drying, samples from each treatment were analysed by GC to determine ACPY retention in comparison with the mixture before spray drying. When ether fractions extracted from the samples were analysed by GC, ACPY and collidine were eluted at about 15.9 and
22 min, respectively. The GC pattern of ACPY and collidine is shown in Fig. 3.

**Stability of ACPY in liquid form**

The losses of ACPY during storage at ambient temperature in acidic and basic solutions are shown in Fig. 4. Higher loss of ACPY during storage occurred in the basic ACPY solution. After 7 days, ACPY reduction was 63% in the basic solution, whereas it was only 30% in the acidic solution after 35 days of storage. These results were consistent with the results reported by Buttery *et al.* (1985) and Rungsardthong (1995). Stable solid salts of ACPY can be prepared by mixing ACPY with an acid (Buttery *et al.*, 1985). The ACPY compound was relatively stable at pH 2. It seemed to dissociate slowly from acids in the sample and showed instability at higher pH (Rungsardthong, 1995).

**ACPY concentration and moisture content after encapsulation**

Statistical analysis showed that ACPY concentrations before and after spray drying were not significantly different (*P* < 0.05) for all the treatments (Table 2). This can be attributed to the stable form of ACPY solution used, confirming
that a stable ACPY salt can be obtained in an acidic solution (Buttery et al., 1985; Rungsard-thong, 1995).

In related research, Desobry et al. (1997) found that drying and encapsulation processes led to an 11% degradation of β-carotene with spray drying. This could be the result of the temperature sensitivity of β-carotene. Sankarikutty et al. (1988) reported some loss of cardamom oil from capsules during spray drying, which occurred because of the extraction of the oil and removal from inside the capsule through cracks.

Results of the moisture analyses showed that the percentage moisture in the spray-dried powders remained between 2 and 4% (Table 2). Rein eccius et al. (1995) found that the percentage moisture in spray-dried gum acacia and maltodextrin powders remained between approximately 2 and 5%. The moisture contents of the product powders with higher ratios of gum acacia were higher, while the moisture content of the products decreased when the amount of maltodextrin increased. Sankarikutty et al. (1988) reported moisture contents ranging from 4.2 to 5.5%, and lower moisture content also occurred in powders with higher ratios of maltodextrin.

ACPY concentration during storage

For encapsulated powders, the ACPY concentration was monitored for a 72-day period (Fig. 5). By using encapsulation techniques, better retention of ACPY was obtained, as shown in Figs 4 and 5. Less than 30% of ACPY reduction was found in all spray-dried samples after 35 days of storage (Fig. 5). These results suggested that encapsulation could be used to extend shelf-stability of ACPY.

In the evaluation of suitable wall materials for ACPY retention, loss in ACPY was observed after 10 days in product powders with 50:50 and 40:60 gum acacia–maltodextrin mixtures (Fig. 5). ACPY content of product powders with 30:70 and 0:100 gum acacia and maltodextrin decreased after 20 days of storage. The 70:30 and 60:40 gum acacia–maltodextrin mixtures had no significant loss in ACPY until 30 days. After 72 days of storage, maximum ACPY reduction, 43.2%, occurred in the product powders of 50:50 gum acacia–maltodextrin and a minimum reduction of 27.7% occurred in the 70:30 gum acacia–maltodextrin mixture. The amount of ACPY in product powders of gum acacia–maltodextrin of 60:40, 40:60, 30:70 and 100% maltodextrin decreased by

Figure 3 Gas chromatographic pattern of ACPY (at 15.9 min) and collidine (at 22 min) from ether fractions extracted from samples.

Figure 4 ACPY reduction during storage in acidic and basic ACPY solutions.
33.4, 35.7, 30.6 and 32.6%, respectively, during the 72 days of storage.

The results showed that the ratio of gum acacia and maltodextrin affected ACPY-flavour retention. Other authors have reported similar results. Thenevet (1995) reported that the orange oil in spray-dried encapsulated powders was stable against oxidation in pure gum acacia and in gum acacia/maltodextrin blends. A 1:1 ratio of acacia gum to maltodextrin yielded a flavour in powder form that had almost the same stability as found when pure acacia gum was used as the carrier. Reineccius (1991) mentioned that maltodextrins typically do not result in good retention of volatile compounds during the spray drying. Reineccius et al. (1995) found that the addition of maltodextrin to traditional acacia shortens the shelf-life of the spray-dried flavour. Orange peel oil encapsulated in an appropriate gum acacia would have a shelf-life of well over 1 year at room temperature without any anti-oxidant.

Pure gum acacia would result in good flavour retention but the cost of the product would be higher. Therefore, the use of maltodextrins decreases the cost of the carrier and allows spray drying at higher feed solid contents because of the lower viscosity of the emulsion (Thenevet, 1995).

From these results, the appropriate wall material for ACPY was a 70:30 gum acacia–maltodextrin mixture. This result is consistent with the observations of Sankarikutty et al. (1988) that the maximum retention of oil after spray drying was obtained with two different combinations of gum acacia and maltodextrin (70:30 and 60:40). The 70:30 combination of gum acacia and maltodextrin gave the best quality capsules in cardamom oil microencapsulation.

### Conclusions

The compound ACPY isolated from pandan leaves by steam distillation extraction had a similar mass spectrum at 5.33 min in GC-MS analysis to that of authentic ACPY.

The ACPY in basic solution decreased by 63% after 7 days, whereas only 30% of ACPY reduction was found in acidic solution after 35 days. ACPY reduction rate was lower in the acidic form.

The ACPY concentrations before and after spray drying were not significantly different (P < 0.05). The percentage moisture in the spray-dried powders remained between 2 and 4% and lower moisture contents occurred in powders with higher ratios of maltodextrin.

### Table 2

<table>
<thead>
<tr>
<th>Gum acacia: maltodextrin</th>
<th>ACPY retention (μg g⁻¹ dry weight)*</th>
<th>Moisture content after spray drying† (%)</th>
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<tbody>
<tr>
<td></td>
<td>Before spray drying</td>
<td>After spray drying</td>
</tr>
<tr>
<td>70 : 30</td>
<td>33.13 ± 1.97a</td>
<td>31.64 ± 0.23a</td>
</tr>
<tr>
<td>60 : 40</td>
<td>36.57 ± 0.33b</td>
<td>35.82 ± 0.41b</td>
</tr>
<tr>
<td>50 : 50</td>
<td>37.68 ± 0.62c</td>
<td>37.88 ± 1.06c</td>
</tr>
<tr>
<td>40 : 60</td>
<td>35.37 ± 0.58d</td>
<td>35.79 ± 0.24d</td>
</tr>
<tr>
<td>30 : 70</td>
<td>34.27 ± 0.37e</td>
<td>34.87 ± 0.34e</td>
</tr>
<tr>
<td>0 : 100</td>
<td>33.46 ± 1.01f</td>
<td>34.84 ± 1.66f</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Average of two replications.
†Average of three replications. Mean values with the same subscript in each row are not significantly different (P < 0.05) by Duncan’s multiple range test.
After 72 days of storage, minimum reduction (27.7%) in ACPY content occurred in 70:30 gum acacia–maltodextrin powder. The amount of ACPY in 60:40, 50:50, 40:60, 30:70 and 0:100 gum acacia–maltodextrin powders decreased by 33.4, 43.2, 35.7, 30.6 and 32.6%, respectively, during 72 days of storage.

References


