Use of spray-cooling technology for development of microencapsulated capsicum oleoresin for the growing pig as an alternative to in-feed antibiotics: A study of release using in vitro models

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ABSTRACT: The aim of this study was to develop sustained release microspheres of capsicum oleoresin as an alternative to in-feed additives. Two spray-cooling technologies, a fluidized air bed using a spray nozzle system and a vibrating nozzle system placed on top of a cooling tower, were used to microencapsulate 20% of capsicum oleoresin in a hydrogenated, rapeseed oil matrix. Microencapsulation was intended to reduce the irritating effect of capsicum oleoresin and to control its release kinetics during consumption by the animal. Particles produced by the fluidized air bed process (batch F1) ranged from 180 to 1,000 μm in size. The impact of particle size on release of capsaicin, the main active compound of capsicum oleoresin, was studied after sieving batch F1 to obtain 4 formulations: F1a (180 to 250 μm), F1b (250 to 500 μm), F1c (500 to 710 μm), and F1d (710 to 1,000 μm). The vibrating nozzle system can produce a monodispersive particle size distribution. In this study, particles of 500 to 710 μm were made (batch F2). The release kinetics of the formulations was estimated in a flow-through cell dissolution apparatus (CFC). The time to achieve a 90% dissolution value (T90%) of capsaicin for subbatches of F1 increased with the increase in particle size (P < 0.05), with the greatest value of 165.5 ± 13.2 min for F1d. The kinetics of dissolution of F2 was slower than all F1 subbatches, with a T90% of 422.7 ± 30.0 min. Nevertheless, because CFC systems are ill suited for experiments with solid feed and thus limit their predictive values, follow-up studies were performed on F1c and F2 using an in vitro dynamic model that simulated more closely the digestive environment. For both formulations a lower quantity of capsaicin dialyzed was recorded under fed condition vs. fasting condition with 46.9% ± 1.0 vs. 74.7% ± 2.7 for F1c and 32.4% ± 1.4 vs. 44.2% ± 2.6 for F2, respectively. This suggests a possible interaction between capsaicin and the feed matrix. Moreover, 40.4 ± 3.9% of the total capsaicin intake in F2 form was dialyzed after 8 h of digestion when feed had been granulated vs. 32.4 ± 1.4% when feed had not been granulated, which suggests that the feed granulation process could lead to a partial degradation of the microspheres and to a limitation of the sustained release effect. This study demonstrates the potential and the limitations of spray-cooling technology to encapsulate feed additives.

Key words: capsaicin, capsicum oleoresin, microencapsulation, spray cooling

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INTRODUCTION

In response to the European ban on in-feed antibiotics in January 2006, the animal feed industry is actively searching for alternatives to improve livestock perfor-
Natural capsaicin is available in liquid form as capsicum oleoresin, a highly irritating product that has limited direct usage as in-feed additive for livestock diets. Controlling the biopharmaceutical behavior of capsaicin in the digestive environment is necessary to ensure optimum use. Although spray-drying techniques afford some protection for capsaicin, the high water solubility values of the carriers used cannot control its release into the gastrointestinal tract. For this reason, another technique was required. The microencapsulation technology by spray cooling presented here could be able to decrease the irritant effect of this oleoresin and to control its release throughout the gastrointestinal tract in a cost-effective way.

The aim of these experiments was to study the release kinetic of capsaicin from microspheres produced by spray-cooling technology. Initially, an in vitro dissolution technique described in the European pharmacopeia was used to discriminate the formulations produced. However, the media used in these techniques are very simple. Thus, we decided to also use a more realistic in vitro model.

**MATERIALS AND METHODS**

Animal Care and Use Committee approval was not obtained for this study because no animals were used.

**Materials**

Rapeseed hydrogenated oil (Loders Croklaan, Wormerveer, the Netherlands) and oleoresin of capsicum with 6% of capsaicin (Capsicum frutescens L.; C. annum L. var., Ernesto Ventos S.A, Spain) were used as a matrix and active compounds, respectively, in preparation of microspheres.

**Production of Microspheres**

Two technologies of spray cooling were used to produce the microspheres, a fluidized air bed using a spray nozzle (F1) and a system with a vibrating nozzle placed on top of a 5-m cooling tower (F2). In the first method, the oleoresin of capsicum (200 kg) was added to 800 kg of molten hydrogenated rapeseed oil (1.2% of capsaicin in the final product) and stirred by an anchor stirrer at 80°C. Microspheres were prepared in a fluidized air bed using an MP11 (Aeromatic Fielder AG, Bubendorf, Switzerland) with a Delavan SDX SJ, Orifice Disc 703-110, 1-component nozzle. The main functional parameters were pump pressure (6.5 bars), product temperature (0 to 4°C), and spraying rate (8 kg/min). As the final product has a large size distribution, we sieved it to select 4 subbatches: F1a (180 to 250 μm), F1b (250 to 500 μm), F1c (500 to 710 μm), and F1d (710 to 1,000 μm).

In the second technique, the oleoresin of capsicum (240 g) was added to 960 g of molten, hydrogenated rapeseed oil and stirred by an anchor stirrer at 80°C. The melt was sieved through a 24-μm metal sieve (Linker Industrie-Technik GmbH, Kassel, Germany). The liquid was gently pumped through a vibrating nozzle system (Brace GmbH process, Alzenau, Germany) in which the fluid stream breaks up into uniform droplets upon exiting. A monodispersive, grain size distribution can be obtained with a constant flow though the nozzle. The amplitude and frequency of nozzle oscillation was maintained constant by a closed-loop, control circuit, but both can be varied to a degree to influence grain size. A melting unit with a 5-m cooling tower (~40°C) was used for drip-casting with a 200-μm, 8-fold nozzle plate. A pressure of 500 mbar was chosen, and the temperature of the heating chambers was set at 80°C. For this study, a batch F2 with particle size between 500 to 710 μm was used.

**Characterization of Microspheres**

Physical tests were performed to evaluate the microsphere characteristics. Size was determined on 100 g of sphere, using sieves of 90, 125, 180, 250, 355, 500, 710, 800, 1,000, and 1,400 μm, and a jel 200 vibratory shaker (Retsch GmbH, Haan, Germany) for 10 min. The surface appearance of the microspheres was assessed directly by stereomicroscopy (Nikon SMZ 1000, Nikon France, Champigny sur Marne, France).

The dissolution test was carried out according to the US Pharmacopeia (USP 26) and European Pharmacopeia (2003) methods, using a flow-through cell, on 1 g of each formulation in a solution imitating the gastric and intestinal contents of a growing pig (details in Meunier et al., 2006). One milliliter of sample was collected at 5, 10, 20, 30, 40, 60, 90, 120, 135, 150, 165, 180, 240, 300, 360, and 480 min.

**Dynamic Gastric-Small Intestinal System**

A dynamic multicompartmental computer-controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals was used. Briefly, the TNO in vitro model (TIM) is composed of 4 successive compartments simulating the stomach, duodenum, jejunum, and ileum (Minekus et al., 1995). Each compartment is composed of glass units with flexible inside walls. The system is kept at body temperature by pumping water into the space between the glass jacket and the flexible wall. Peristaltic mixing is simulated by alternate compression and relaxation of the flexible walls following changes in the water pressure. Mathematical modeling of gastric and ileal deliveries with power exponential equations \( f = 1 - 2^{-\beta}t^{t_{1/2}} \), where \( f \) represents the fraction of meal delivered, \( t \) is the time of delivery, \( t_{1/2} \) is the half-time of delivery, and \( \beta \) is a coefficient describing the shape of the curve) is used for the computer control of chyme transit, as described by Elashoff et al. (1982) and modified by Decuyper et al. (1986). This in vitro
model has previously been used to study the behavior of orally administered drug dosage forms simulating various human gastrointestinal conditions (Blanquet et al., 2004).

In the current study, the system was programmed to reproduce the physiological conditions in the gastrointestinal tract of a growing pig, according to in vivo data (Minekus 1998). The half-time of gastric emptying and ileal delivery were fixed at 150 and 650 min, respectively, whereas the $\beta$ coefficient of the power exponential equation was fixed at 1 and 2.17. Chyme transit was regulated by opening or closing the peristaltic valves that connect the compartments. The volume and pH was computer-monitored and continuously controlled in each compartment. In the stomach, the pH followed a preset curve, with a pH of 6.0, 3.5, 3.0, 2.5, and 2.0 at 5, 30, 120, 180, and 240 min, respectively, by addition of 1 M HCl. In the small intestine, the pH was maintained at 5, 6.5, and 6.5 in the duodenum, jejunum, and ileum, respectively, by addition of 1 M NaHCO$_3$. Simulated gastric and pancreatic secretions were introduced into the corresponding compartments by computer-controlled pumps. Water and small molecules (i.e., products of digestion and dissolved capsaicin) were removed by pumping dialysis liquid (20 mL/min) through hollow fiber membrane units (cut off = 5,000 Da; HG 600, Hospal Cobe, Lyon, France) connected to the jejunal and ileal compartments. As for the dissolution test, the behavior of capsaicin oleoresin in TIM was determined by the dosage of capsaicin.

**Chemicals.** Pepsin A from porcine stomach mucosa (2,100 units/mg, P-7012), trypsin from bovine pancreas (7500 BAEE units/mg, T-4665), pancreatin from porcine pancreas (P1750), and porcine bile extract (B-8631) were all purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Lipase from Rhizopus lipase (150,000 units/mg, F-AP 15) was purchased from Amano Enzyme Inc. (Nagoya, Japan).

**Diet and Solutions Used in the Model.** To evaluate additive-feed interactions, capsaicin oleoresin availability was studied when it was administered with a stomach electrolyte solution (fasted condition) or a feed (fed condition). A commercial, standard corn diet composed of corn (54%), soybean meal (28%), and barley (15%) was used (as-is basis; see ingredients and analysis in Anguita et al., 2007). Feed was administered in meal or in granulated form (temperature of 70°C and die of 4/20 to obtain pellets of 4 mm). The additive was added to the diet before granulation, and in both cases it included 15% of microencapsulated oleoresin of capsicum and 85% of the commercial, standard corn diet (as-is basis).

The solutions used were saliva, 152 TAU/L of alpha-amylase (Spezyme AA Genencor International B.V., Leiden, the Netherlands) in distilled water; stomach compartment, HCl (1 mol/L), stomach electrolyte (3 g/L of NaCl, 1.1 g/L of KCl, 0.15 g/L of CaCl$_2$, and 0.6 g/L of NaHCO$_3$, 3 g/L of SDS), pepsin (0.21 g/L of stomach electrolyte), and lipase (0.25 g/L of stomach electrolyte) solutions; and duodenal compartment, NaHCO$_3$ (1 mol/L), intestinal electrolyte (0.6 g/L of KCl, 5.0 g/L of NaCl, 0.23 g/L of CaCl$_2$·2H$_2$O, 3 g/L of SDS), pancreatin (100 g/L), bile (40 g/L), and trypsin (2 g/L) solutions.

Because the active compound (oleoresin of capsicum) used was poorly soluble in water, 0.3% of SDS was used as surfactant in all media used in the digestion studies.

**Experimental Protocols**

Six protocols were carried out to study the factors of interest affecting capsaicin release. Every protocol was tested in the model during triplicate 8-h experiments. For experiments performed without feed, samples from each compartment (stomach, duodenum, jejunum, ileum) and from jejunum and ileum dialyses were collected every hour and analyzed. In this study, luminal availability was defined as the total amount of capsaicin dissolved in the jejunum and ileum compartments and in the 2 dialyses. For experiments performed with feed, only samples from jejunum and ileum dialysis were analyzed.

**Protocol I.** To study the behavior of free capsiicum oleoresin (not microencapsulated) in the TIM in fasted conditions, 1.5 g of capsiicum oleoresin was mixed with a stomach electrolyte solution (298.5 mL) and used as a test meal. All enzymes and bile secretions were replaced by intestinal electrolyte solutions (0.5 and 1.0 mL/min in the stomach and the duodenum, respectively).

**Protocol II.** To study the impact of microencapsulation on the release of capsiicum oleoresin in the TIM in fasted conditions, 6 g of microspheres was mixed with stomach electrolyte solution (294 mL) and used as a test meal. The secretions used were the same as for protocol I.

**Protocol III.** To study the impact of feed on the release of microencapsulated capsiicum oleoresin in the TIM, 40 g of feed A was mixed with stomach electrolyte solution (260 mL) and used as a test meal. The secretions used were the same as for protocol I.

**Protocol IV.** To study the impact of enzyme on release of microencapsulated capsiicum oleoresin in the TIM, 40 g of feed A was mixed with the saliva solution (260 mL) and used as a test meal. The lipase and pepsin solution were secreted in the stomach at 0.25 mL/min. To mimic a physiological situation of residues left from previous meals, the stomach was filled with residue at the beginning of the experiments with 5 mL of each solution. The pancreatin solution was secreted at 0.25 mL/min, and bile secretion was simulated by secreting a 4% bile solution at 0.5 mL/min. Before the experiment, the duodenal compartment was filled with 1 mL of trypsin solution (2 mg/mL) + 14 mL of bile solution + 7.5 mL of pancreatin solution, and 7.5 mL of small intestinal electrolyte solution (NaCl, 5 g/L; KCl, 0.6 g/L; and CaCl$_2$, 0.23 g/L).

**Protocol V.** To study the impact of feed granulation on the release of microencapsulated capsiicum oleoresin
in the TIM, 40 g of feed B was mixed with stomach electrolyte solution (260 mL) and used as a test meal. The secretions used were the same as for protocol I.

**Protocol VI.** To study the impact of enzyme on the release of microencapsulated capsicum oleoresin in feed granulated in the TIM, 40 g of feed B was mixed with stomach saliva solution (260 mL) and used as test meal. The secretions used were the same as for protocol IV.

**Analyses**

In all cases, the percentage of capsicum oleoresin recovered was estimated from the recovered amount of capsaicin.

For microspheres, 1 g was placed in 10 mL of acetone. The solution was stirred for 30 min (80 rpm using a TR-225, Infors AG, Bottmingen, Switzerland), centrifuged (5,000 \( \times g \)), and then filtered (0.45-\( \mu \)m pore size) before analysis. Capsaicin was determined by GLC, with a flame ionization detector (GC-FID) by using a Hewlett-Packard HP 6890 (Agilent Technologies, Massy, France) fitted with a capillary column (HP-5, 5\% phenyl methyl siloxane of 30-m \( \times 0.32\) mm ID, 0.25-\( \mu \)m film thickness, Interchrom, Montluçon, France). Temperature was programmed at a rate of 5°C/min from 100 to 150°C and 20°C/min from 150 to 300°C. Detector and injector temperatures were set at 250°C, and the helium gas flow rate was 3 mL/min.

For the dissolution test and for TIM samples, analysis was done directly by HPLC. In the case of samples from TIM, they were centrifuged (5,000 \( \times g \)) and then filtered (0.45-\( \mu \)m pore size) before analysis by HPLC. The method used was an Elite Lachrom Merck Hitachi HPLC (Merck France, Nogent-sur-Marne, France). The column was a UP5HDO-25Qs (C18 5 \( \mu \). - 250 \( \times 4.6 \) mm, Interchrom) with a mobile phase composed of water-acetonitril-acetic acid (55/44.5/0.5, vol/vol/vol) with a flow rate of 1 mL/min. The injection volume was fixed at 20 \( \mu \)L, and the detection wavelength at 280 nm.

The concentration of capsicum oleoresin present in feed was analyzed using extraction with a Soxhlet apparatus (Buchi B811, Flawil, Switzerland). Ten grams of weighed feed was placed in 200 mL of acetone. The solution was heated at 76°C for 4 h (15 cycle), and the sample was then centrifuged (5,000 \( \times g \)) and filtered (0.45-\( \mu \)m pore size) before analysis by GLC with the same method used for the microspheres.

**Statistical Methods**

Values are presented as means ± SD. Comparisons between formulations were performed using a Student’s t-test. All statistical analyses were computed using SAS (SAS Inst. Inc., Cary, NC). Dissolution curves were compared with a model-independent approach using a similarity factor (\( f_{1/f2} \)) previously explained in Meunier et al. (2006).

**RESULTS**

**Production of Microspheres**

The amount of capsaicin recovered from the microspheres was not different for the 2 technologies used (98.5 ± 0.7 for F1 and 98.8 ± 1.4 for F2, \( n = 3 \)). The stereomicroscopic appearance of the microspheres is presented in Figure 1 (panels A and B). The surface of batch F1 was not totally homogeneous because small particles were stuck on the surface of the larger particles. This could be explained by the fluidization of particles in the tower during the process before their discharge. A continuous discharge should improve the product. Alternatively, a lower process temperature inside the tower should be applied to solidify the particles immediately. In contrast, the surface of batch F2 was perfectly homogeneous. The temperature applied during the F2 process was very low (−40°C), and the particles were not in a fluidized air bed but circulating 1 way in a tube and were immediately discharged after solidification. In the case of batch F1, the microspheres obtained had a large size distribution from 180 to 800 \( \mu \)m with more than 77% between 350 and 710 \( \mu \)m. For batch F2 the microspheres were more homogeneous with a more narrow size distribution with 97% between 500 and 710 \( \mu \)m (Figure 2, panels A and B). The vibrating nozzle technology allows better control of the particle size than the spray nozzle, but both spray-cooling techniques gave satisfactory results for the general aspect of the microspheres.

**In Vitro Dissolution**

Because microspheres in the current study were a matrix core, the release of active compounds may depend on how long they diffuse within the matrix and hence on particle size. The impact of this parameter was studied on particles from subbatches F1a, F1b, F1c, and F1d. The second main parameter controlling active compound release is the technology used, so microspheres obtained by the vibrating nozzle system were compared with microspheres of the same size, F1c (500 to 710), obtained by the fluidized bed technique. Figure 3 shows dissolution profiles of subbatches obtained from F1, and Table 1 shows the times for 50% (\( T_{50\%} \)) and 90% dissolution (\( T_{90\%} \)). Results indicate a delay in release kinetic with an increase in particle size (\( P < 0.05 \)). The f1/f2 test was used to compare the dissolution profile of each formulation. A difference (\( P < 0.05 \)) was observed (f1 > 15 and f2 < 50) in capsaicin release profiles between all F1 subbatches (only 1 measurement was considered after 85% of dissolution). The difference was restricted from 40 to 90 min between F1c and F1d.

The dissolution \( T_{50\%} \) and \( T_{90\%} \) were greater for F2 than for all F1 subbatches, being around 67 and 422 min, respectively (\( P < 0.05 \)). The dissolution profile was different (\( P < 0.05 \)) between F2 and all F1 subbatches. With F1d this difference was significant only from 15
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Figure 1. Stereomicroscopy of microsphere (20% of capsiicum oleoresin into a rapeseed, hydrogenated oil matrix). A) F1 formulation produced by fluidized air bed using a spray nozzle; B) F2 formulation produced by the vibrating nozzle system put on top of a 5-m cooling tower.

Figure 2. Granulometry of microsphere (20% of capsiicum oleoresin into a rapeseed, hydrogenated oil matrix; n = 3). A) F1 formulation produced by fluidized air bed using a spray nozzle; B) F2 formulation produced by the vibrating nozzle system put on top of a 5-m cooling tower.

The fluidized bed system. A lower temperature can shorten the crystallization process and also modify the structure of the microspheres, which could explain the difference in the release kinetic profiles obtained with the F2 formulation.

Digestion Using Dynamic Gastric-Small Intestinal System

Initially, we evaluated the feasibility of the process by the digestion of free capsicum oleoresin (not microencapsulated) in TIM without any secretion (protocol I). The aim was to check that capsicum oleoresin transit accurately followed the preset computer-monitored curves to simulate the transit of chyme in the model. The gastric delivery and intestinal transit of capsaicin, expressed as a percentage of the total intake, are presented in Figure 4 (panels A and B, respectively). Curves representing the quantity of capsaicin dissolved in the stomach or available in the intestinal compartments (luminal availability) after intake of free oleoresin of capsicum were not different from the preset curves simulating stomach delivery and intestinal tran-
Figure 3. Dissolution profiles of capsaicin from microspheres of capsicum oleoresin (20% of capsicum oleoresin into a rapeseed, hydrogenated oil matrix). The F1 formulation was produced by fluidized air bed using a spray nozzle and sieved to obtain 4 formulations: F1a (180 to 250 μm), F1b (250 to 500 μm), F1c (500 to 710 μm), and F1d (710 to 1,000 μm). The F2 formulation was produced by the vibrating nozzle system put on top of a 5-m cooling tower. Error bars represent SD (n = 6). Legend: A) F1a ( ), F1b ( ▲ ), F1c ( ▼ ), F1d ( ● ); and B) F2 ( ), means ± SD. *Within a time, means with different superscript letters are different (P < 0.05). For F1 subbatches, the difference factor (f1)/similarity factor (f2) test identified significant difference between dissolution profiles, this difference is restricted from 40 to 90 min between F1c and F1d dissolution profiles. The similarity factor f1/f2 test indicated significant difference between F2 and all F1 subbatches dissolution profiles.
Table 1. Time to achieve 50 and 90 dissolution (T50% and T90%, respectively), means ± SD

<table>
<thead>
<tr>
<th>Formulation1</th>
<th>T50%, min ± SD (n = 6)</th>
<th>T90%, min ± SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1a (90 to 355 μm)</td>
<td>4.7 ± 1.0a</td>
<td>18.5 ± 6.5a</td>
</tr>
<tr>
<td>F1b (355 to 500 μm)</td>
<td>8.7 ± 0.5b</td>
<td>38.3 ± 4.0b</td>
</tr>
<tr>
<td>F1c (500 to 710 μm)</td>
<td>16.5 ± 0.8c</td>
<td>105.8 ± 10.7c</td>
</tr>
<tr>
<td>F1d (710 to 1,000 μm)</td>
<td>29.5 ± 1.2d</td>
<td>168.5 ± 13.2d</td>
</tr>
<tr>
<td>F2 (500 to 710 μm)</td>
<td>67.5 ± 2.0e</td>
<td>422.7 ± 30.3e</td>
</tr>
</tbody>
</table>

a–eColumn values with different superscripts differ (P < 0.05).

1The F1 formulation was produced by fluidized air bed using a spray nozzle and sieved to select 4 size ranges of microspheres, with F1a (180 to 250) μm, F1b (250 to 500) μm, F1c (500 to 710) μm, and F1d (710 to 1,000) μm subbatches. The F2 formulation was produced by the vibrating nozzle system put on top of a 5-m cooling tower.

Afterwards, the impact of microencapsulation on capsaicin release kinetics was studied (protocol II). The quantities of capsaicin dosed in the stomach compartment expressed as a percentage of the total intake are presented in Figure 5 and were compared with results obtained from the digestion of free oleoresin of capsicum. The microencapsulation of capsicum oleoresin with F1c formulation delayed the gastric dissolution of capsaicin. The values obtained were similar to those obtained for free capsicum oleoresin intake only at 120 min, which means that all the capsaicin had been delivered from the microspheres at this time. With the F2 formulation, release was longer because it took between 240 and 300 min for all capsaicin to be delivered. The amount of capsaicin available in the intestinal compartment, expressed as a percentage of the total intake, is presented in Figures 6 and 7. The microencapsulation of oleoresin of capsicum with F1c did not delay the delivery of capsaicin. The concentration of capsaicin in the intestinal compartment of TIM was not different from that in the study with free capsaicin oleoresin (P > 0.05) with 87.3% ± 1.9 and 87.2% ± 4.3, respectively, of total intake after 480 min. In contrast, F2 formulation delayed the delivery of capsaicin, and only 59.1% ± 2.9 of the capsaicin intake was dissolved in the small intestine at 480 min (P < 0.05). These results were confirmed by values in the dialysis fraction, with 76.2% ± 1.6 and 74.7% ± 2.7 of the total intake of capsaicin being recovered in the dialysis after 480 min for the studies with free oleoresin of capsicum and F1c formulation, respectively, vs. 44% ± 1.6 for the F2 formulation (Figure 7).

The following step was to study the impact of feed on release kinetics of microencapsulated capsicum oleoresin (protocol III). Because the amount of free capsaicin in a feed matrix is difficult to determine, this analysis was not carried out only in the dialysis samples. Results obtained reflect the quantity of capsaicin dissolved and dialyzed. The quantities of capsaicin in dialysis expressed as a percentage of the total intake are presented in Figure 8 for F1c formulation and in Figure
Figure 5. Average concentration of free capsaicin in the gastric compartment of a dynamic multicompartmental computer-controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from 3 formulations: free capsicum oleoresin, an F1c formulation produced by fluidized air bed using a spray nozzle and sieved to obtain particle size between 500 to 710 μm, and an F2 formulation produced by vibrating nozzle system put on top of a 5-m cooling tower (particle size between 500 to 710 μm). Error bars represent SD (n = 3). Legend: theoretical value (■), experience with free capsicum oleoresin (▲), experience with F1c formulation (●), experience with F2 formulation (◆). a–cWithin a time, means with different superscript letters are different (P < 0.05).

In protocol V and VI the effect of the granulation of the feed on the release of capsaicin was studied. Results were compared with digestion of nongranulated feed (Figure 10). The amount of capsaicin dialyzed at 480 min was greater with granulated feed than with nongranulated feed (P < 0.05) 40.4% ± 3.9 vs. 32.4% ± 1.4. As in the previous study, addition of enzyme and bile secretion did not modify the kinetics of absorption by dialysis (P > 0.05) with 37.7% ± 3.1 of capsaicin absorbed after 480 min of granulated feed digestion.

DISCUSSION

Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is a major pungent hydrophobic alkaloid of capsicum fruits (i.e., chili pepper and paprika) that presents interesting effects for animal production. Initially capsaicin was studied due to its antimicrobial activity (Ciuchewicz and Thorpe, 1996), but other properties have been studied such as the ability to simulate digestive enzyme secretion from pancreas and bile production from the liver (Bhat et al., 1984; Platel and Srinivasan, 1996, 2000, 2004), to induce a greater retention time of the diet (Platel et al., 2002), and to increase gastrointestinal blood flow (Leung, 1993). This increase in the retention time and irrigation has been proposed to improve HCl secretion and nutrient digestion and absorption (Dunshea, 2003).

Capsaicin can be obtained directly from ground spices, but use of the oleoresin form is more hygienic, easily standardized, more concentrated in capsaicin, and thus requires less storage space. However, oleoresin is a highly irritant product, which limits its direct use as a microadditive for livestock diets and, in this liquid form, is sensitive to light, heat, and oxygen, and has a short storage life if not stored properly. Microencapsulation protects oleoresin against such destructive changes and also converts it into a free-flowing powder that can be easily and homogeneously mixed with feed,
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Figure 6. Average cumulative quantity of capsaicin into intestinal compartment of a dynamic multicompartmental computer-controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from 3 formulations: free capsicum oleoresin, an F1c formulation produced by fluidized air bed using a spray nozzle and sieved to obtain particle size between 500 and 710 μm, and an F2 formulation produced by vibrating nozzle system put on top of a 5-m cooling tower (particle size between 500 and 710 μm). Error bars represent SD (n = 3). Legend: free oleoresin of capsicum (▲), F1c formulation (●), F2 formulation (◆). a,bWithin a time, means with different superscript letters are different (P < 0.05).

which makes administration easier. Moreover, microencapsulation can control the biopharmaceutical behavior of the additive in the gastrointestinal tract. Control of capsaicin release at a specific site of action in the gastrointestinal tract could improve its efficiency. The main aim of this study was to suggest formulations with different release profiles of the capsicum oleoresin to evaluate their impact on in vivo studies on performance of growing pigs.

Microencapsulation of capsicum oleoresin can be achieved by spray drying in some polysaccharide matrices (Zilberboim et al., 1986; Xiang et al., 1997; Jung and Sung, 2000). Although spray drying affords a certain degree of protection for capsaicin, the high water solubility of the carrier used cannot control its release into the gastrointestinal tract. Moreover, the concentration of capsicum oleoresin that can be encapsulated by spray drying is very low, and the product produced is generally a fine powder that is an irritant product, which limits its direct use as a microadditive. For these reasons, another technique was required for the controlled release. Most of these techniques are difficult to apply directly in the feed industry due to cost and legislation problems, so developing a control release formulation that meets all requirements of the feed industry in terms of cost and safety is a challenge.

Spray-cooling technology seems able to answer this challenge. The matrix used in this technology to produce microspheres is hydrophobic (hydrogenated oil wax), which can create a barrier between the active compound and an aqueous medium like the digestive environment.

To establish the biopharmaceutical properties of feed additives using in vivo methods, especially those based on the use of surgically modified animals, requires special facilities, and the methods are expensive, time-consuming, and are increasingly subject to ethical objection. On the other hand, in vitro models are relatively inexpensive, rapid, reproducible, and easy to perform. Therefore, we used a dissolution apparatus classically used for quality control but also important in characterizing the biopharmaceutical quality of a product. Of these apparatuses, the flow-through cell is the best adapted to the study of microspheres (Meunier et al., 2006). Using this method we determined that from the subbatches selected from F1, nonsustained release was observed with a small particle size (less than 500 μm). With larger particle size, sustained release was observed, and this effect was greater with the largest size. These results confirm that particles size is a key parameter that should be controlled to develop sustained release of microspheres.

Using the second technology (vibrating nozzle) evaluated in this study, we could produce monodispersing particles between 500 to 710 μm similar to batch F1c, but the dissolution T90% was increased. The matrix composition and the concentration of active compound were equal in both technologies; only the type of nozzle
Figure 7. Average cumulative quantity of capsaicin dialyzed into a dynamic multicompartmental computer-controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from 3 formulations: free capsicum oleoresin and an F1c formulation produced by fluidized air bed using a spray nozzle and sieved to obtain particle size between 500 and 710 μm, and an F2 formulation produced by vibrating nozzle system put on top of a 5-m cooling tower (particle size between 500 and 710 μm). Error bars represent SD (n = 3). Legend: free oleoresin of capsicum (■), F1c formulation (●), F2 formulation (◆). a–cWithin a time, means with different superscript letters are different (P < 0.05).

Figure 8. Average cumulative quantity of capsaicin dialyzed into a dynamic multicompartmental computer-controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from 2 formulations: free capsicum oleoresin and an F1c formulation produced by fluidized air bed using a spray nozzle and sieved to obtain particle size between 500 and 710 μm, in fasted and fed condition. Error bars represent SD (n = 3). Legend: total capsaicin dialyzed (●), F1c formulation without feed (◆), F1c formulation with feed (■), F1c formulation with feed and enzymes (▲). a–cWithin a time, means with different superscript letters are different (P < 0.05).
Figure 9. Average cumulative quantity of capsaicin dialyzed into a dynamic multicompartmental computer controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from 2 formulations: free capsicum oleoresin and an F2 formulation produced by the vibrating nozzle system put on top of a 5-m cooling tower, in fasted and fed condition. Error bars represent SD (n = 3). Legend: total capsaicin dialyzed (●), F2 formulation without feed (◆), F2 formulation with feed (■), F2 formulation with feed and enzymes (▲). a–c Within a time, means with different superscript letters are different (P < 0.05).

Figure 10. Cumulative concentration of capsaicin dialysis in the dynamic multicompartmental computer controlled model developed by TNO Nutrition and Food Research (Zeist, the Netherlands) that simulates the functions of the stomach and the small intestine function of monogastric animals, expressed as a percentage of total intake from F2 formulation produced by the vibrating nozzle system put on top of a 5-m cooling tower, in fasted and fed condition, in fed condition with feed granulated and not granulated. Error bars represent SD (n = 3). Legend: an F2 formulation with feed (●), an F2 formulation with granulated feed (■), an F2 formulation with granulated feed and enzyme secretion (▲). a,b Within a time, means with different superscript letters are different (P < 0.05).
and the temperature used during the process to solidify the matrix were different. A lower temperature applied to solidify the particle could modify the structure of the matrix and have an impact on its dissolution property.

Despite the usefulness of the dissolution test, the media used in this technique are still very simple and do not simulate the complex influences of the feed matrix on formulations or the gradually changing conditions during each step of digestion related to the digestive fluids, presence of the meal, and absorption of nutrients. However, these parameters can influence the solubility of the active compound in the gastrointestinal tract (Dressman et al., 1998). This is why, in a second step, we chose a more realistic in vitro method to study the biopharmaceutical behavior of microencapsulated capsicum oleoresin. The TIM could be a useful tool in feed additive studies to determine where and when a compound is released; what might influence its release, its stability, and its availability for absorption; and what role the presence of feed, transit time, enzymes, or formulation could play in these processes (Blanquet et al., 2004). The study with TIM showed that microspheres from F2 formula had a greater delay in capsaicin release than particles F1. This result could be explained by the experimental conditions used with the vibrating nozzle system and in particular the process temperature (−40°C) applied. However, applying a very low temperature during the microencapsulation process to produce control release microspheres involves an extra cost that should be considered depending on the additive to be encapsulated.

The studies performed with the TIM detected no effect of digestive secretions on release of capsicum from the microspheres. In contrast, use of TIM showed a clear action of feed matrix with a decrease in the capsaicin dialyzed. This effect could be the consequence of interactions between the formulation or the active compound and the feed. Finally, this in vitro system demonstrated that the process of microsphere incorporation into feed followed by a granulation process could lead to a partial destruction of the microspheres and so to a limitation of the sustained release effect. This result underlines the limits of spray-cooling technology to control active compound release in feed additives.

These results demonstrate the advantages and some limitations of spray-cooling technology to control active compound release in feed additives. We still need to conduct in vivo experiments, but this technology seems to be able to produce control-release microspheres of capsicum oleoresin in a cost-effective practical way. This technology should be considered for other additives to avoid detrimental effects, like irritating or bad taste effects, and to achieve site-specific effects by modifying some parameters such as the size of the particle. These studies also show the usefulness of the TIM as an important tool to test in-feed additives in a realistic way.

LITERATURE CITED


Cichewicz, R. H., and P. A. Thorpe. 1996. The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. J. Ethnopharmacol. 52:61–70.


