Microencapsulated supercritical carbon dioxide extract of small cardamom enriches the nutraceutical value of custard
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The spray dried α-amylase-assisted supercritical carbon dioxide (SC-CO₂) extract of small cardamom (rich in 1,8-cineole) was used for the fortification of custard to obtain a new designer nutraceutical custard. The encapsulate was employed at concentrations of 4%, 4.5% and 5% (w/w) to formulate the custard. Custard formulated with 4.5% of encapsulate was most preferred by the sensory panel. This custard sample showed a more stable texture, higher phytochemical properties and lower microbial load than the control sample (formulated without encapsulate). This is the first study on the formulation of a new designer nutraceutical custard by fortification with encapsulated SC-CO₂ extract of small cardamom. The processes described here can safely be employed in the development of other nutraceutical or functional foods, which inevitably involves the loss of the phytochemicals of food constituents during manufacture.

Keywords
Custard
Small cardamom
α-Amylase-assisted SC-CO₂ extract
Spray drying

Introduction
The in vitro phytochemical and antimicrobial properties of supercritical carbon dioxide (SC-CO₂) extracts of Alleppey Green small cardamom seeds (Elettaria cardamomum (L.) Maton) have been previously described by our research group [1]. The authors have employed the 1,8-cineole-rich extract of small cardamom obtained in the above study in the formulation of custard [1]. However, when the custard was evaluated for its nutraceutical value, it did not possess the same phytochemical potency as that of the extract, indicating a possible loss of phytochemicals (31.15% loss of total phenolic content) during custard formulation.

To redress the phytochemical loss during custard preparation, enzyme (α-amylase)-assisted SC-CO₂ extraction of small cardamom extract was performed. The 1,8-cineole content of the enzyme (α-amylase)-assisted SC-CO₂ extract of small cardamom was 29.55% higher than that of the extract obtained by SC-CO₂ extraction alone [2]. To avoid further decrease in the nutraceutical value of custard, the present study formulated custard using the enzyme-assisted SC-CO₂ extract in microencapsulated (spray dried) form. Spray drying of this extract using maltodextrin and gum arabic as wall materials (70:30) at an inlet air temperature of 130°C effectively preserved the 1,8-cinole content and antioxidant potency of the extract [2].

The objectives of this study were therefore: (a) formulation of an antioxidant-fortified custard using the spray dried enzyme-assisted 1,8-cineole-rich SC-CO₂ extract of small cardamom for minimization of loss of the nutraceutical property of the custard; and (b) characterization of the newly formulated custard for its sensory, physicochemical and phytochemical properties. To the best of our knowledge, there is no literature on the fortification of a dessert with encapsulated SC-CO₂ extract of small cardamom for the formulation of a new designer nutraceutical product. The processes described here can safely be extended to design new nutraceutical or functional foods, whose manufacture inevitably involves a decrease in the phytochemical potency of their constituents.

Materials and methods

Materials
Seeds of Alleppey Green small cardamom (Elettaria cardamomum (L.) Maton) were procured from Spices Board,
Cochin, India. Specialty chemicals such as α-amylase from Bacillus licheniformis (lyophilized powder, 500–1,500 units/mg protein, 93–100% SDS-PAGE), 1,8-cineole (99% pure), 1,1-diphenly-2-picrylhydrazyl (DPPH), sodium nitroprusside, maltodextrin (dextrose equivalent 16.5–19.5), gum arabic and gallic acid were procured from Sigma (St Louis, MO, USA). Silica gel 60 (F254)-coated Al plates, Folin-Ciocalteu’s phenol reagent (FCR), methanol, ethanol, sodium carbonate and 0.45 μm filter papers were purchased from E-Merck (Mumbai, India). Food grade CO2 was procured from BOC India (Kolkata, India). All chemicals used in this work were of AR grade. Custard powder (Weikfield Products, Pune, India) was obtained from a local supermarket in Kolkata.

α-Amylase-assisted SC-CO2 extraction of 1,8-cineole-rich extract from small cardamom

The α-amylase-assisted SC-CO2 extraction of the 1,8-cineole-rich extract of small cardamom seeds was conducted using SCF Green Technology SPE-ED SFE 2 equipment (Applied Separations, Allentown, PA, USA). A 20 g sample of ground cardamom seeds (dp=0.42±0.02 mm) was mixed with lyophilized α-amylase (in the previously optimized ratio of 5000:1) and subjected to SC-CO2 extraction at 200 bar, 50°C and 135 min extraction time (120 min static time+15 min dynamic time) with a 2 l/min flow rate of gaseous CO2 [2].

Our investigations have revealed that α-amylase-assisted SC-CO2 extraction enhanced the yield of extract from small cardamom by 50.66% and the concomitant yield of 1,8-cineole in the extract by 29.55% compared to extractions performed without enzyme [2]. The extract was subsequently spray dried using a Mini Spray Dryer B-290 (Buchi, Flawil, Switzerland). A full factorial design was used to optimize the spray drying conditions using maltodextrin: gum arabic ratios of 80:20, 70:30 and 60:40 for wall material composition and inlet air temperatures of 110°C, 130°C and 150°C.

A 1 g sample of 1,8-cineole-rich small cardamom extract was used for each experiment. Compressed air at a pressure of 8 bar was used for spray drying. The spray gas flow rate, gas flow rate in the aspirator, sample feed rate and atomization pressure were kept constant at 473 l/h, 35,000 l/h, 9.0 ml/min and ~60 mbar, respectively, for all experiments. The optimized conditions for spray drying were found to be an inlet air temperature of 130°C and wall material composition of maltodextrin: gum arabic of 70:30. The encapsulate obtained at these conditions had the highest 1,8-cineole content and antioxidant properties along with the highest microencapsulation efficiency [2].

The encapsulate thus obtained in the experiments was designated as the best encapsulate (Ebest). This Ebest was employed in designing a nutraceutical custard.

Formulation of custard with Ebest

Custard samples were prepared in accordance with the method developed in our laboratory by Chatterjee et al. [3], with modifications. First, 3 g custard powder and 5 g ground sugar were added to 50 ml boiling milk with continuous stirring. Ebest was then added and the mixture was cooked at 75±3°C for 2 min. Different concentrations of Ebest (4%, 4.5% and 5%, w/w of custard) were used for the preparation of different custard samples (C4, C4.5 and C5, respectively; see Fig. 1) with the aim of obtaining nutraceutical fortified custard samples. A custard sample prepared without the encapsulate served as control (Ccontrol). The samples were cooled to room temperature (23±2°C) after cooking. All custard samples were stored at 4±1°C in autoclaved screw-capped glass jars (50 ml) before analysis.

Microbiological analysis of custard samples

The total plate count (TPC) of custard samples was determined for both bacteria and fungi using the pour plate method, immediately after preparation. Samples (1 ml) of diluted custard were poured on to nutrient agar and potato dextrose agar plates for estimation of TPCs for bacterial and fungal growth, respectively. The plates were incubated at 37±1°C for 24 h for bacteria and at 25±1°C for 72 h for fungi. After incubation, TPCs for bacteria and fungi were determined as CFU/g custard.

Figure 1 - Custard samples: (a) Ccontrol; (b) C4; (c) C4.5; (d) C5
Sensory evaluation of custard samples
Sensory evaluation of the custard samples was conducted by a semi-trained panel of university faculty members and research scholars (10 men and 10 women) aged 20-45 years. The panellists were selected based on their interest and performance in screening tests conducted with a control sample, and were familiar with the sensory attributes of custard. Samples were served in glass bowls with stainless steel spoons. All the samples were blind coded using three-digit numbers and served randomly to the panellists. The panelists used the standard 9-point hedonic scale to evaluate the custard samples (9 indicating ‘like extremely’ and 1 indicating ‘dislike extremely’) on the attributes of overall appearance, colour, odour, texture, taste and aftertaste. The sensory evaluation was conducted between 10 am and 12 noon in a well-ventilated room under white light [4]. A rest period of 5 min between consecutive samples was allowed to minimize sensory fatigue. Unsalted crackers and water were provided to panellists to rinse their palate before each evaluation [5]. The individual samples were served in triplicate in each session and rounded off mean scores were represented graphically by radar plots [6]. C4.5 achieved the highest sensory scores. Therefore, the physicochemical and phytochemical properties of C4.5 and Ccontrol were determined.

Estimation of the physicochemical properties of custard samples
pH of custard samples
The pH of custard samples was estimated according to the method reported by Chatterjee and Bhattacharjee [7]. A 5 g custard sample was homogenized with 25 ml deionized water and pH was measured using a PC 510 pH meter (Eutech Instruments, Singapore).

Analysis of colour
The colour of custard samples was measured using a Hunter Lab colorimeter (Konica Minolta, Tokyo, Japan) at a 10° inclination from the light source and reported as L*, a* and b* values. The colour co-ordinates of the custard samples were calibrated against a standard white plate. Chroma values and hue angles were calculated using standard equations [7].

Rheology study
The rheological behaviour of custard samples was assessed using a Modular Compact Rheometer (MCR) 102 (Anton-Parr, Graz, Austria) with a cone and plate (CP-40) type arrangement (having clearance of 0.08 mm between the two components) at 23±2°C. The flow behaviour of the samples was measured in controlled shear rate mode. The shear rate was varied from 0.0001 to 100/s. The small deformation of storage modulus (G') and loss modulus (G”) were recorded. Amplitude sweeps with varying amplitude from 0.01% to 100% were conducted to study the changes in G’ and G” at a constant angular frequency of 10 rad/s. Frequency sweeps were also conducted with angular frequencies ranging from 0.1 to 100 rad/s at a constant amplitude of 0.5%. G’ and G” were measured separately as functions of amplitude and frequency.

Chemical analyses of nutraceutical properties of custard samples
For estimation of the 1,8-cineole content and phytochemical properties of the custard samples, such as antioxidant activity, total phenolic content and reducing power, 1 g of custard sample was dissolved in 10 ml ethanol. The 1,8-cineole contents of the samples were estimated using high performance thin layer chromatography (HPTLC) according to the method described by Patra et al. [8], with few modifications. The amount of 1,8-cineole present in the custard samples was determined from the standard curve prepared with standard 1,8-cineole (Rf=0.58±0.01 at λmax of 1,8-cineole=500 nm).

The loss of 1,8-cineole in the C4.5 sample during preparation of the custard was estimated by comparing the 1,8-cineole content of C4.5 with that of the encapsulate (Ebest).

The antioxidant activities of the custard samples were determined by measuring the radical scavenging activity of DPPH [9] and were expressed as IC50 values (mg/ml). The total phenolic content was estimated using Folin-Ciocalteu’s reagent [10] and expressed as mg gallic acid equivalent/g of custard. The reducing power determined as mg BHT/g of custard was estimated according to the method of Oyaizu [11]. The total phenolic content and reducing power of small cardamom extracts were estimated from their respective standard curves of gallic acid and BHT, respectively.

Results and discussion
Microbiological analysis of custard samples
The TPCs of Ccontrol were found to be 18 CFU/g custard for bacteria and 4 CFU/g custard for fungi, while the TPCs of C4.5 were 8 CFU/g custard for bacteria and 2 CFU/g custard for fungi; both values of C4.5 were lower than those of the control set. According to the guidelines of the Food Safety and Standards Authority of India [12], the highest permissible aerobic plate count in custard is 1×10³ CFU/g sample. Therefore, the C4.5 custard sample is microbiologically safe for human consumption. This is in agreement with the fact
that small cardamom extract reportedly contains antimicrobial properties [1], which could have possibly contributed to the reduction in microbial load in the C4.5 custard sample.

Sensory evaluation of custard samples prepared with Ebest

The panellists awarded equal scores for overall appearance to Ccontrol, C4, C4.5 and C5 (Fig. 2). The characteristic yellow colour of the custard samples was sensorially approved by all panellists. The panellists moderately liked the characteristic odour of Ccontrol. The pleasant and sweet aroma of small cardamom was strongest in C5, which was therefore most preferred by the panel, followed by that of C4.5 and C4. C4.5 received the highest scores for its smooth texture, followed by C5, C4 and Ccontrol. Panellists liked the taste of Ccontrol moderately, while among the custards fortified with cardamom encapsulate, C4.5 was liked most because of its sweet taste, followed by C4. The taste of C5 was slightly bitter and disliked by the panel. The distinct sweet aftertaste of small cardamom was perceived in C4.5, which made it more appealing than C4 and Ccontrol. The aftertaste of C5 was slightly pungent and was disliked by the panellists. Overall, C4.5 was judged the best by the panellists. Therefore, further physicochemical, phytochemical and microbiological analyses were conducted for C4.5, along with Ccontrol.

Estimation of the physicochemical properties of custard samples

pH of custard samples

Ccontrol (7.00) and C4.5 (7.02) had identical pH. Thus, addition of cardamom encapsulate did not affect the pH of newly formulated custard.

Analysis of colour

The colour of the custard samples was represented by L*, a*, b*, chroma and hue angles (Table 1). Ccontrol and C4.5 had a light (high L* values) yellowish (high b* values) colour. The chroma values of the samples indicated that both samples had equally bright intensities. The hue angles (84.09±0.02° and 84.13±0.01°) indicated the light-yellowish colour of the samples, in agreement with that reported by our research group for mayonnaise prepared with eugenol-lean SC-CO2 extract of clove buds [7]. The C4.5 and Ccontrol samples had a yellow colour (Table 1), confirming that the addition of the encapsulate did not have an adverse effect on the appearance of the custard samples.

Rheology study

The flow curves of Ccontrol and C4.5 indicated pseudo-plastic or shear-thinning flow behaviour for both samples (Fig. 3a,b), in agreement with the results obtained by Kėsienė et al. [13], who examined the effect of milk fat and tapioca starch on the rheological properties of dairy custards and on the release of strawberry flavour compounds therein. The G’ and G” values denote the viscoelastic behaviour of the samples against applied stress. Analysis of rheograms for the amplitude sweep of the samples showed that for both samples, the values of G’ were well above those of G”, indicating strong thickening and solidifying behaviour (Fig. 3c,d) in both. In the Ccontrol sample, G’ and G” crossed over at 39.8% strain, and at 63.1% strain in the C4.5 sample. Therefore, the viscoelastic region of the C4.5 sample was higher than that of the Ccontrol sample, indicating the broad viscoelastic nature of C4.5. This finding was in agreement with the sensory scores where the smooth and consistent texture of C4.5 was most preferred by the panellists.

In frequency sweeps (Fig. 3e,f), no crossover of G’ and G” values was observed in either sample, attesting to the stability of the viscoelastic nature of the custard in the frequency range investigated. For C4.5, G’ and G” values showed insignificant changes with frequency, and G’ was greater than G” throughout the frequency range studied for custard (0.1–100 rad/s). These observations indicate a desirable strong cohesive association within the custard and its consequent firmness.

Estimation of the nutraceutical properties of custard samples

Application of a 1,8-cineole-rich encapsulate to custard significantly enhanced its phytochemical properties (Table 2). Ghosh et al. [1] observed a 31.15% loss of total phe-
Table 1 - Analysis of colour of the Ccontrol and C4.5 samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccontrol</td>
<td>81.86±0.11</td>
<td>4.34±0.01</td>
<td>43.74±0.13</td>
<td>43.98±0.19</td>
<td>84.09±0.02</td>
</tr>
<tr>
<td>C4.5</td>
<td>81.82±0.13</td>
<td>4.47±0.03</td>
<td>43.49±0.19</td>
<td>43.72±0.17</td>
<td>84.13±0.01</td>
</tr>
</tbody>
</table>

Chroma=√(a* 2 + b* 2); hue angle=tan⁻¹(b*/a*)
The L*, a*, b*, chroma and hue angle values of custard samples are the means±SD of three independent samples.

Table 2 - 1,8-Cineole content and phytochemical properties of custards

<table>
<thead>
<tr>
<th>Sample</th>
<th>1,8-Cineole content of custard (mg/g custard)</th>
<th>IC50 of DPPH radical scavenging activity (mg/ml)</th>
<th>Reducing power (μg BHT/g custard)</th>
<th>Total phenolic content (μg GAE/g custard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccontrol</td>
<td>NA</td>
<td>5.37±0.05b</td>
<td>9.01±0.03a</td>
<td>2.01±0.01a</td>
</tr>
<tr>
<td>C4.5</td>
<td>1.12±0.05</td>
<td>2.04±0.02a</td>
<td>41.28±0.07b</td>
<td>8.19±0.04b</td>
</tr>
</tbody>
</table>

1,8-Cineole content, IC50 of DPPH radical scavenging activity, reducing power and total phenolic content values are the means±SD of three independent custard samples. Different letters in a column indicate significant difference at p≤0.05. NA not applicable.

nolic content in custard (since 27.9 μg gallic acid content of extract enhanced the phenolic content of 1 g custard by 19.21 μg gallic acid). However, in our formulation, the application of encapsulated extract minimized the loss of total phenolic content and 1,8-cineole content to 1.59% (since 6.28 μg gallic acid content of encapsulate enhanced the phenolic content of 1 g custard by 6.18 μg gallic acid) and 1.23% (1.134 mg 1,8-cineole content of powder contributed to 1.120 mg 1,8-cineole content of 1 g custard), respectively. These findings indicate higher nutraceutical potency of custard prepared with encapsulated extract than of that prepared with the extract per se, establishing the protective role of microencapsulation in preventing the degradation of phytochemicals (in SC-CO2 extract of small cardamom) utilized in custard formulation. In addition, the encapsulate did not adversely affect the physicochemical properties and sensory attributes of the newly formulated custard. Therefore, custard fortified with spray dried extract of small cardamom is a new designer nutraceutical (functional) dessert.

**Conclusion**

Spray dried powder from α-amylase-assisted SC-CO2 extract of small cardamom was added to custard at 4%, 4.5% and 5% (w/w) levels to enhance its nutraceutical properties. Custard formulated with 4.5% encapsulate was most liked sensorially. This custard sample also exhibited better rheological stability, higher phytochemical potency and lower microbial load than its control. The C4.5 sample is therefore a nutraceutical-rich dessert. Similar applications of spray dried enzyme-assisted SC-CO2 extract of small cardamom could be envisaged for other desserts and spreads.

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Human and animal rights
This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of interest
The authors declare that they have no conflict of interest.

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