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Development of liquid-core hydrogel beads to improve hydration in elderly: Pilot studies

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ABSTRACT

This study aimed to develop liquid-core hydrogel beads (LHBs) using spherification technique to promote increased water intake in the elderly. Orange juice was selected as the encapsulation liquid due to its widespread availability and consumer acceptance. The formulation included calcium lactate (1% w/v), xanthan gum (0.025% w/v), and sodium alginate (0.5% w/v). Quality parameters such as size, texture, pH, total soluble solids, antioxidant activity, glycemic index and microbial safety were determined. The hydrogel beads maintained physical homogeneity for up to 3 days, while microbiological safety was ensured for at least 7 days under 4°C. In a trial involving 30 elderly participants, each of whom consumed nine beads (50 mL each) daily, 77% indicated high sensory acceptability and reported improved hydration and increased fluid intake. The findings from this study offer valuable insights for caregivers and healthcare providers working with the elderly.

1. Introduction

The global aging population presents significant health and social challenges, with the proportion of people aged 60 and older expected to be nearly doubled from 12 % in 2015 to 22 % by 2050. The population of older adults, which stood at 1 billion in 2019, is projected to reach 1.4 billion by 2030 and 2.1 billion by 2050 (Yin et al., 2024). As the elderly population grows, addressing their health needs, particularly issues related to nutrition and hydration, is a critical concern (Klojdova et al., 2025; Volkert et al., 2019). Dehydration is a common problem among elderly due to physiological changes such as reduced muscle mass, decreased kidney function, difficulties with swallowing, and a diminished thirst response. Recent meta-analytical data suggests that nearly 24 % of older adults suffer from dehydration when evaluated by directly-measured serum or plasma osmolality (Parkinson et al., 2023). In addition, dehydration in older adults can lead to a range of adverse health conditions, including constipation, falls, delirium, renal failure, infections, and even mortality (Thomas et al., 2008).

Proper hydration is critically necessary for supporting functions such as temperature regulation, waste elimination, and joint lubrication (Benelam & Wyness, 2010; Jéquier & Constant, 2010; Lorenzo et al., 2019). However, managing hydration in the elderly requires more than simply increasing water intake. As mentioned above, the elderly often face challenges with swallowing, which complicates their ability to consume sufficient fluids (de Sire et al., 2022; Sura et al., 2012). Other problems include diminished taste sensation, which may be associated with poor oral and dental health, zinc deficiency, comorbidities, or the side effects of medications (Solemdal et al., 2012).

Given the unique challenges faced by the elderly, innovative solutions are needed to ensure effective hydration. Although there are commercially available products which aim to prevent dehydration in the elderly, including electrolyte powders and easy to swallow fluids, their taste and required preparation steps make them inconvenient to use. To overcome these limitations, one possible way forward is to develop jelly-like products which can be easily orally processed and swallowed. Previous studies have demonstrated the suitability of such systems for safe ingestion. For example, Aii et al. demonstrated the usefulness of sliced gelatin jelly that can pass through the pharynx as a single cohesive bolus without collapsing (Aii et al., 2024). Cho et al. reported that swallowing-aid jelly mixed with beverages could safely

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deliver medications without affecting their therapeutic efficacy (Cho et al., 2024). Similarly, Patel et al. developed instant jelly powders to encapsulate sustained-release drugs, with the jelly matrix acting as a carrier that controlled drug release (Patel et al., 2020). Although these formulations differ in composition and application, their works demonstrate the potential of jelly-based formulations as safe carriers for key ingredients, suggesting their applicability in addressing dehydration in the elderly.

Jelly-based formulations can be produced in various sizes and textures, and can also be personalized if necessary, ensuring both ease of use and palatability. However, their production must prioritize shelf life and comply strictly with food safety standards, especially since elderly individuals are often immunocompromised. Furthermore, these products must also be designed to meet the recommended daily water intake, which is at least 1.6–2.0 L per day for older adults (Masot et al., 2020).

In this research, reverse spherification technique was employed to produce liquid-core hydrogel beads (LHBs). Unlike conventional small spherification products typically used for culinary or pharmaceutical applications, these beads are designed to deliver a substantial volume of fluid while remaining easy to chew and swallow. The LHB formulation was optimized to produce large size beads with a thin membrane. We hypothesized that this design could maintain shape stability while effectively retaining the functional properties of the encapsulated liquid. In addition to hydration, the product formulation can also incorporate other health functional food elements such as phytochemicals and antioxidants. Although spherification has been widely studied (Bennacef et al., 2021; Oupathumpanont et al., 2025; Zou et al., 2024), its application to hydration products remains underexplored. By addressing both hydration requirements and age-related swallowing difficulties, this study aims to develop and characterize LHBs as a new class of hydration products for elderly.

2. Experimental

2.1. Materials

Food-grade quality sodium alginate (viscosity; 600–800 cPs, 1 wt % in water at 20°C), calcium lactate (CL), and xanthan gum (viscosity; 1200–1800 cPs, 1 wt % in KCl solution (1 wt %) at 20°C) were procured from Chemipan Co., Thailand. Distilled water was used to prepare all reagents and solutions. Oranges (*Citrus tangerina*) were obtained from a local market and subsequently washed with distilled water. The fruits were then peeled and sliced. The orange pulp was macerated using a hand blender and filtered through cheesecloth. The resulting juice was pasteurized at 75°C for 15 min to inactivate microorganisms and enzymes. After pasteurization, the juice was stored at 4°C until further use.

2.2. Preparation of liquid-core hydrogel beads (LHBs)

The core solution was prepared by dissolving calcium lactate 10 g in orange juice 1 L to form a solution 1 % (w/v). Xanthan gum (250 mg) was then stirred into the solution until it completely dissolved to give a concentration of 0.025 % (w/v). The orange juice mixture was poured into spherical mold (55 mm inside diameter) and stored in a freezer overnight at -20°C (Fig. S1). Sodium alginate bath at a concentration of 0.5 % w/v was prepared by adding 10 g of sodium alginate in boiling water (2 L), and cooled to 4°C for 24 h before spherification. Once the orange juice mixture was frozen, it was removed from the molds and soaked in the sodium alginate bath for 16 min, followed by rinsing with distilled water. The prepared hydrogels were stored in pure water at 4°C for subsequent physicochemical and clinical evaluation (Fig. S2).

2.3. Characterization of LHBs

2.3.1. Dimension, wall thickness and sphericity

The diameter and gel membrane thickness of LHBs were measured using a vernier caliper. For measuring the gel membrane thickness, the LHBs were cut open to remove the internal liquid, and the coating membrane was washed with water before its thickness was measured. Sphericity of LHBs was calculated according to Eq. 1.

$$\text{Sphericity} = \frac{2d_{min}}{(d_{min} + d_{max})} \quad (1)$$

where d_{min} is the minimum diameter and d_{max} is the maximum diameter of the LHBs. Values closer to 1 indicate a higher degree of sphericity.

2.3.2. Color, total soluble solid, pH and texture analysis

Color analysis was performed on a single LHB from each formulation using a HunterLab spectrophotometer (UltraScan Pro, HunterLab, USA). Three replicates of each sample were randomly selected for analysis. The Reflectance Specular Excluded mode was used, specifically designed for semi-solid samples with glossy surfaces (Bubin et al., 2019).

Total soluble solids and pH were measured directly using a portable digital refractometer (Model: MSDR-P2-102, IMS Euro Ltd. Stockport, England) and a digital pH meter (Model: SevenDirect SD23, Mettler Toledo, USA), respectively.

The texture of LHBs was determined using a TA-XT Plus texture analyzer (Stable Micro Systems, England) with a spherical probe (P/0.5, 5.0 mm diameter). The compression test was conducted with pre-test, test, and post-test speeds set at 1.0, 5.0, and 5.0 mm/s, respectively, and a distance of 40 cm.

2.3.3. Loading capacity (LC) and encapsulation efficiency (EE)

LHBs were weighed and then cut open to weigh internal liquid. Loading capacity (LC) of LHBs was calculated according to Eq. 2 while encapsulation efficiency (EE) was determined following Eq. 3 which is based on the difference between the weight of the core solution before formulation and weight of the core solution after formulation (the internal liquid).

$$\text{LC}\ (\%) = \frac{(\text{Measured core solution weight},\ \text{g})}{(\text{Weight of LHBs},\ \text{g})} \times 100 \quad (2)$$

$$\text{EE}\ (\%) = \frac{(\text{Measured core solution weight},\ \text{g})}{(\text{Initial weight of core solution},\ \text{g})} \times 100 \quad (3)$$

2.4. Release test by UV–Vis spectroscopy

The release of internal components from the LHBs into the surrounding medium was evaluated using a UV–Vis spectrophotometric method. The beads were stored in deionized water, and the surrounding solution was sampled at Days 0, 1, 3, and 7. At each time point, 1 mL of the storage solution was collected and diluted with deionized water to a final volume of 3 mL to ensure consistent optical measurements. The absorbance spectra were recorded in the wavelength range of 320–700 nm using a UV–Vis spectrophotometer (Spectroquant® Prove 100, Merck) equipped with 1-cm path-length quartz cuvettes. The increase in absorbance at 326 nm, a wavelength characteristic of carotenoids present in orange juice, was used as an indicator of component release from the LHBs.

2.5. Determination of antioxidant activities

2.5.1. The ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) assay was adapted from a method previously described by Somsong *et al* (Somsong et al., 2020). Briefly, the hydrogel samples were extracted using a 1:1 v/v ratio of dimethyl sulfoxide (DMSO). The FRAP reagent was prepared using a 10:1:1 mixture of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$. Aliquot solutions were used as experimental samples, with 20 µL of each aliquot placed in a 96-well plate. Subsequently, 200 µL of the FRAP reagent was

added, mixed thoroughly, and incubated at 37°C for 30 min. The absorbance was measured at 593 nm. Trolox was used as a standard, with dilutions plotted at various concentrations to generate a calibration curve using the FLUOstar Omega software (BMG Labtech, Germany). Results were expressed as μmol of Trolox equivalent per mL of extracted solution.

2.5.2. Oxygen radical absorbance capacity (ORAC)

The antioxidant activity was measured using the oxygen radical absorbance capacity (ORAC) assay employing a spectrophotometric technique according to a methodology published previously with a few modifications (Somsong et al., 2020). Briefly, LHB samples were extracted using a 1:1 v/v ratio of dimethyl sulfoxide (DMSO). Aliquots of 20 μL from the extracted samples were placed into a 96-well plate. An auto-injection system was employed to add 160 μL of 120 nM fluorescein, followed by 20 μL of 480 μM AAPH. All solutions were prepared using phosphate-buffered saline (PBS, 75 mM, pH 7.0). Trolox served as the standard control, with dilutions plotted based on the area under the curve at various concentrations. The calibration curve was generated using the FLUOstar Omega software (BMG Labtech, Germany). The excitation wavelength was set to 485 nm and the emission wavelength to 528 nm. Results were expressed as μmol of Trolox equivalents per mL of extracted solution.

2.6. Total phenolic and vitamin C content

The total phenolic content of the samples was measured following the method described by Wispen et al (Wispen et al., 2022). Each sample was initially added 25 μL to the 96-well plate. Subsequently, 50 μL of diluted (1:10) Folin-Ciocalteu reagent (2 N) was added and the mixture was thoroughly mixed for 5 min. Then, 200 μL of 7.5 % (w/v) Na_2CO_3 was added, followed by additional mixing. The reaction mixture was incubated in the dark at room temperature for 2 h, and the absorbance was measured at 765 nm. A standard calibration curve was prepared using gallic acid at concentrations ranging from 3.125 to 200 μg/mL, and the results were expressed as milligrams of gallic acid equivalents per 100 mL (mg GAE/100 mL). Vitamin C content was estimated using ascorbic acid test strip (MQuant®, Merck) and expressed as mg/L (ascorbic acid).

2.7. Determination of in vitro glycemic index

The estimated glycemic index (eGI) was calculated following the method described by Chaipai *et al* (Chaipai et al., 2018). Briefly, 100 mg of jelly LHBs were weighed, and 10 mL of HCl-KCl buffer (pH 1.5) were added, followed by 0.2 mL of HCl-KCl buffer (pH 1.5) containing 57.9 μmol of pepsin. The samples were incubated in a shaking water bath at 40°C for 60 min (WNE22 with shaking device, Memmert, Germany). Afterward, 9 mL of 0.1 M Tris-maleate buffer (pH 6.9) and 1 mL of α-amylase were added to each sample, and incubation continued at 37°C in a shaking water bath for 0, 20, 30, 60, 90, and 120 min. The reaction was stopped by placing the tubes in ice, in order to inactivate the α-amylase. Samples were then centrifuged at 4000 rpm for 10 min at 25°C. To prepare the sample mixture, 1 mL of the supernatant was combined with 500 μL of 100 % ethanol. Glucose concentration was determined using the glucose oxidase-peroxidase (GOPOD) kit. 3 mL of GOPOD reagent were placed in a brown bottle, along with 100 μL of the sample mixture, and incubated for 20 min in a 40°C water bath. A 250 μL sample was then placed in a 96-well plate, and the absorbance was measured at 510 nm using a microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, Vermont, USA). The area under the curve was analyzed for glucose release using GraphPad Prism version 5.01 (GraphPad Software, CA, USA).

2.8. Microbial analyses

The microbial testing of the LHBs was conducted in accordance with the Notification of the Ministry of Public Health (No. 416) B.E. 2563 (2020), issued under the authority of the Food Act B.E. 2522 (1979). The following methods were used for microbial analysis: the most probable number (MPN) of coliforms and *Escherichia coli* was determined using FDA BAM Online (2020, Chapter 4); yeast and mold counts were assessed using BAM Online (2001, Chapter 18); *Salmonella* spp. was tested according to ISO 6579:2002; and *Staphylococcus aureus* was analyzed using BAM Online (2001, Chapter 12).

2.9. Sensory evaluation and consumer acceptance

The LHBs were assessed for sensory attributes by an elderly panel consisting of 30 semi-trained judges, using a 5-point Hedonic scale to evaluate parameters such as color, aroma, taste, ease of consumption, and overall acceptability. The mean scores of 30 semi-trained judges were used to evaluate the quality of the LHBs. The study was conducted with approval from the Institutional Review Board (IRB). Protocol No. MU-CIRB 2022/191.1207. COE No. MU-CIRB 2022/126.1108

2.10. Clinical evaluation

The LHB was introduced in a nursing home or hospital setting to assess its acceptability and efficacy in promoting hydration and water intake among the elderly. The study panel consisted of male and female participants over the age of 60 who were able to speak, read, communicate, and understand Thai, and who agreed to participate for the duration of the study. Thirty individuals were involved in the research, each evaluating one product. The food tasting test and preference rating took approximately 20 min to complete.

Dehydration in the elderly was assessed through self-perceived symptoms, including dry mouth, before and after consuming the LHB. This was evaluated using a 3-item questionnaire focused on frequency of urination, thirst, and dry mouth, referred to as the Council of Nutrition Appetite Questionnaire (Wilson et al., 2005). The study was conducted with Institutional Review Board (IRB) approval. Protocol No. MU-CIRB 2022/191.1207. COE No. MU-CIRB 2022/126.1108

2.11. Statistical analysis

All values are presented as mean values with standard deviation of triplicate determinations. Data analysis was carried out using PASW Statistics 18.0 (SPSS Inc, Chicago, IL, USA). The results were compared by one-way ANOVA followed by Tukey's analysis. A significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Optimization of LHBs formulation

In the initial experiments, LHBs were prepared using freshly squeezed orange juice. The orange juice was selected as the core liquid for encapsulation due to its widespread availability and consumer acceptability. Additionally, it serves as an excellent source of polyphenols and vitamin C (de Paiva et al., 2019). The orange juice was pasteurized at 75°C for 15 min to kill pathogenic bacteria. This low temperature pasteurization was used to preserve quality of citrus juices (Kumar et al., 2023). Calcium lactate (CL) and xanthan gum were then added into orange juice. In this case, calcium lactate was selected as calcium salts because it does not give any unpleasant taste compared to calcium chloride (Lovera et al., 2014) and give a faster spherification rate than calcium gluconate (Lee & Rogers, 2012). The addition of xanthan gum, thickening agent, improves texture and stabilize both aroma and flavor of LHB (Akkarachaneeyakorn & Tinrat, 2015).

The reverse spherification method was adapted from a previous study (de Farias & Zapata Noreña, 2019). During the optimization phase in small scale (orange juice 1 L), the concentration of CL was fixed at 1 % (w/v), while sodium alginate (SA) varied between 0.5–0.8 % (w/v). Xanthan gum was maintained at a constant concentration of 0.025 % (w/v). These levels were selected based on preliminary sensory test, as they represented the minimum concentration required to preserve the orange juice while maintaining a desirable flavor profile.

The mixture was filled in spherical molds and kept frozen at -20°C in a freezer overnight. To evaluate the effect of immersion time on membrane thickness, the frozen beads were placed in an alginate bath for durations ranging from 12 to 20 min. The results are summarized in Table 1. Our findings indicate that both the concentration of the SA bath and the immersion time significantly impact membrane thickness. The thickness increased as both concentration of SA and immersion time increased. While the 0.8 % SA concentration produced a thicker membrane at any given immersion time, the resulting LHB surface appeared rougher (Fig. S3). This is likely due to the higher density of alginate chains, which provides more carboxyl and hydroxyl groups to bind calcium ions, leading to increased crosslinking and a rougher surface structure (Maleki et al., 2020). Consequently, we selected the 0.5 % SA bath because it consistently produced a smoother surface.

In addition, we aimed for the thinnest membrane to ensure the beads are easy to chew. Although a 12-min immersion time produced the thinnest membrane suitable for the target product, a 16-min immersion time was selected to ensure more complete gelation and improved structural integrity of the beads.

3.2. Effect of storage conditions

To determine appropriate storage conditions for LHBs, the LHBs were stored either in water or without water at 4°C for a period of 7 days (Fig. S2). The orange juice encapsulated inside the LHBs was used as the indicator of storage stability. Changes in the internal juice were monitored throughout the storage period, and the results are summarized in Table 2.

The results showed that LHBs stored in water exhibited only a 13 % loss of internal liquid, whereas a substantially higher loss of 39 % was observed for LHBs stored without water. These findings suggest that the encapsulated liquid gradually diffuses through the membrane over time. However, the presence of surrounding water slows the rate of liquid loss, with the system appearing to reach equilibrium after approximately 3 days.

3.3. Preparation and physical property of LHBs

Based on the initial results, 0.5 % SA, 16-min immersion time and storing in water were used to produce and storge LHBs. However, under same conditions, the membrane thickness reduced to 0.30 mm (Table 3). This unexpected decrease is likely attributable to the variations between batches of orange juice. Differences in orange juice properties, particularly pH and total soluble solids, can strongly affect gelation behavior. Although controlling these parameters is important for ensuring consistent product quality, such control was not feasible within the scope of the present study and therefore represents a limitation. The physicochemical characteristics of the LHBs are summarized in Table 3.

Table 1
Thickness of LHBs by using 0.5 % and 0.8 % SA with 1 % CL at different of times.

Concentration of alginate bath	Time (min)		
	12	16	20
Sodium alginate 0.5 %	0.27±0.06^{a}	0.50±0.00^{b}	0.57±0.06^{b}
Sodium alginate 0.8 %	0.47 ± 0.06^{a}	0.53 ± 0.06^{a}	0.77± 0.06^{b}

Values are presented as mean ± SD (n = 3). Different superscript letters within a row indicate significant differences determined by Tukey's test ($p \leq 0.05$).

Table 2
Orange juice content in LHBs under different storage conditions after 7 days of storage.

Day	Orange juice content (mL)	
	Wet condition	Dry condition
0	53.75 ± 1.15^{c}	54.00 ± 0.00^{c}
1	50.00 ± 0.00^{b}	47.67 ± 2.31^{bc}
3	46.33 ± 1.53^{a}	42.00 ± 4.00^{b}
7	47.00 ± 1.73^{ab}	33.00 ± 4.36^{a}

Values are presented as mean ± SD (n = 3). Different superscript letters indicate significant difference within column by Tukey's test ($p \leq 0.05$).

Table 3
Physical analysis of LHBs.

Parameter	Value
Size (mm)	W 64.00 ± 0.89 L 65.27 ± 0.70 H 26.83 ± 1.65
pH	4.16 ± 0.01
TSS	8.07 ± 0.29
Thickness (mm)	0.30 ± 0.00
Internal liquid (mL)	50.00 ± 0.00
Sphericity	0.58 ± 0.04
%LC	91.00 ± 0.00
%EE	96.15 ± 0.00

Physical properties, including size, pH, total soluble solids, wall thickness, color, and orange juice content, were determined. Representative images of the prepared LHBs are shown in Fig. 1. The LHB measured 65.27 ± 0.70 mm in width, 64.00 ± 0.89 mm in length, and 26.83 ± 1.65 mm in height. Each LHB holds about 50 mL of orange juice. This amount is roughly equivalent to a small glass of water. The size was designed to be conveniently held and consumed, making it both practical and efficient for hydration purposes. The wall thickness was 0.30 mm, which allows for easy chewing, even for those who may use false teeth. The pH of the LHB is an important consideration, as the skin of the elderly tends to be drier and more prone to cracking. The pH of the prepared LHB was 4.16, which is slightly acidic due to the natural properties of orange juice; however, at this pH, they can still be safely used by the elderly (Blaak et al., 2015; Kuo et al., 2020). The total soluble solids measured 8.07. Sphericity was calculated to be 0.58. Loading capacity (LC) and encapsulation efficiency (EE) were 91.00 % and 96.15 %, respectively.

3.4. LHBs stability

The LHB was stored in pure water for 7 days to monitor any physical changes. Initially, the LHB exhibited a homogeneous color without any separation. However, between Days 1 and 3, gradual separation of the juice inside the LHBs was observed, and by Day 7, the juice had completely separated (Fig. S4). By Day 7, the color of the LHBs had lightened compared to Days 0–3, with a higher proportion of red and green hues (Table 4). The observed color changes are associated with oxidation and degradation of orange juice pigments, such as carotenoids and flavonoids, which are sensitive to oxygen, light, and temperature. As shown in Tables 5 and 6, the physical and texture analysis of the LHB revealed that hardness, which indicates their resistance to bursting during production and storage, progressively decreased over time. The results demonstrate that as the storage period increased, hardness, springiness, gumminess, and chewiness declined due to internal leakage from the LHB. This leakage was confirmed using UV–Vis spectroscopy by regularly sampling the surrounding storage solution (Fig. 2). The presence of orange juice in the external medium was indicated by a gradual increase in absorbance at 326 nm, a wavelength associated with carotenoids. This steady rise suggests that the membrane became less

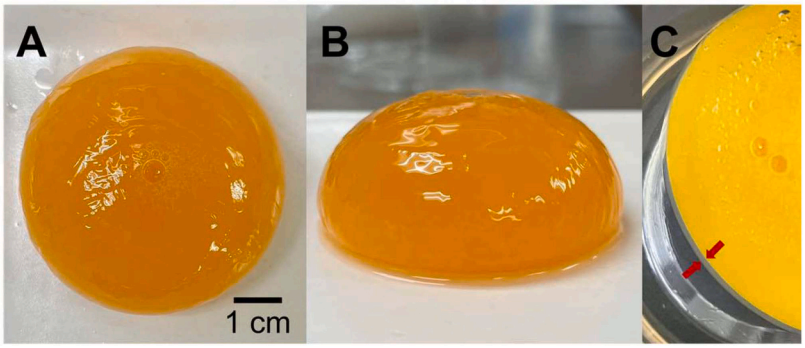

Fig. 1. Image of prepared LHBs: (A) top view, (B) side view and (C) membrane thickness.

Table 4
Color analysis of LHBs at different days.

Day	L*	a*	b*
0	38.04 ± 0.16^{ab}	6.84 ± 0.3^{NS}	30.30 ± 0.56^{a}
1	38.90 ± 0.40^{bc}	7.64 ± 0.53^{NS}	30.69 ± 0.82^{a}
3	37.38 ± 0.25^{a}	7.72 ± 0.80^{NS}	29.07 ± 1.44^{a}
7	40.18 ± 0.86^{c}	8.45 ± 0.80^{NS}	34.07 ± 1.56^{b}

Values are presented as mean ± SD (n = 3). Different superscript letters within a column indicate significant differences by Tukey's test ($p \leq 0.05$), while "NS" indicates no significant difference within the same column.

Table 5
Physical properties of LHB at different days at 4°C.

Day	0	1	3	7
Size (mm) (W x L x H)	65.27 ± 0.70^{c} 64.00 ± 0.89^{c} 26.83 ± 1.65^{NS}	60.93 ± 0.46^{b} 60.37 ± 0.72^{b} 25.77 ± 0.61^{NS}	61.93 ± 0.78^{b} 59.90 ± 0.70^{b} 26.87 ± 1.61^{NS}	58.67 ± 1.03^{a} 57.23 ± 1.27^{a} 28.07 ± 0.49^{NS}
pH	4.16 ± 0.01^{b}	4.15 ± 0.00^{b}	4.15 ± 0.01^{b}	4.09 ± 0.01^{a}
TSS	8.07 ± 0.29^{NS}	8.05 ± 0.30^{NS}	8.43 ± 0.06^{NS}	8.43 ± 0.12^{NS}
Thickness (mm)	0.30 ± 0.00^{NS}	0.30 ± 0.00^{NS}	0.30 ± 0.00^{NS}	0.30 ± 0.00^{NS}
Internal liquid (mL)	50.00 ± 0.00^{b}	49.33 ± 0.58^{b}	48.00 ± 1.73^{ab}	45.67 ± 1.15^{a}
%EE	96.15 ± 0.00^{b}	94.87 ± 1.12^{b}	92.31 ± 3.33^{ab}	87.82 ± 2.21^{a}

Values are presented as mean ± SD (n = 3). Different superscript letters within a row indicate significant differences by Tukey's test ($p \leq 0.05$), while "NS" indicates no significant difference within the same row.

Table 6
Texture analysis of LHB at different days.

Attributes	Day 0	Day 3	Day 7
Hardness (g)^{NS}	222.35 ± 36.26	215.74 ± 36.44	157.06 ± 70.80
Adhesiveness (g sec)^{NS}	16.66 ± 1.89	13.19 ± 1.03	11.98 ± 5.81
Resilience (%)^{NS}	32.06 ± 2.89	34.03 ± 2.48	35.45 ± 0.90
Cohesion (%)^{NS}	64.14 ± 7.51	64.38 ± 2.43	83.53 ± 26.82
Springiness (%)^{NS}	79.75 ± 2.89	73.33 ± 0.47	76.85 ± 4.83
Gumminess^{NS}	14,368.05 ± 3310.73	13,844.83 ± 1821.54	12,169.93 ± 1700.79
Chewiness^{NS}	11,520.53 ± 2921.79	10,156.92 ± 1400.71	9311.21 ± 719.58

Values are presented as mean ± SD (n = 3). Different superscript letters within a row indicate significant differences by Tukey's test ($p \leq 0.05$), while "NS" indicates no significant difference within the same row.

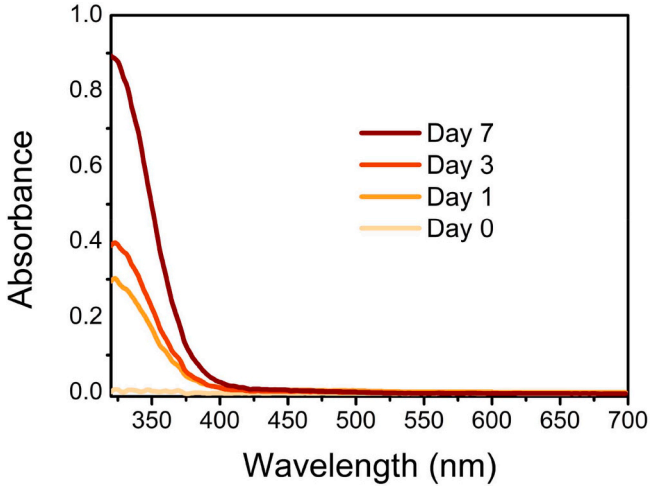

Fig. 2. UV–Vis spectra of the surrounding water of LHBs on different storage days.

effective over time, allowing components from inside the beads to diffuse into the surrounding water. As a result, the beads gradually lost their structural strength and textural integrity during storage.

3.5. Microbial test

The microbial analyses were shown in Table 7. The results showed that microbial loads remained almost unchanged during storage under 4°C. The product was safe for consumers in terms of microbial content, meeting the standards set by the Ministry of Public Health (Announcement No. 416, B.E. 2563) and the Food and Drug Administration. However, although microbial safety was maintained for up to 7 days, changes in bead homogeneity and texture were observed after 3 days suggesting LHBs should be consumed within 3 days after production to

Table 7
Microbial analysis of LHB stored at 4°C.

Bacteria Test	Day 0	Day 1	Day 3	Day 7
MPN coliform/g	Less than 3	Less than 3	Less than 3	Less than 3
MPN *E. coli* /g	Less than 3	Less than 3	Less than 3	Less than 3
Yeast and Mold/g	Less than 10 cfu (estimated count)	Less than 10 cfu (estimated count)	Less than 10 cfu (estimated count)	Less than 10 cfu (estimated count)
Salmonella spp./25 *g*	Not detected	Not detected	Not detected	Not detected
Staphylococcus aureus/0.1 *g*	Not detected	Not detected	Not detected	Not detected

maintain its physical property.

In contrast, when LHBs were stored at 25°C, microbial growth was detected at day 3 (Table S1). These results indicate that higher temperatures promote microbial contamination. Therefore, LHBs are not recommended for storage under ambient conditions and are only suitable for consumption when kept under refrigerated conditions.

3.6. Total phenolic content, vitamin C and antioxidant activity

Total phenolic content, vitamin C and antioxidant activity of LHBs over 7 days were investigated. As shown in Table 8, the total phenolic content of LHBs decreased significantly over the 7-day storage period, from 130.99 ± 11.07 μg GAE/mL on Day 0 to 71.96 ± 1.12 μg GAE/mL on Day 7. This reduction is likely due to the oxidation and degradation of phenolic compounds during storage, even under 4°C. The results suggest that membrane does not completely prevent oxidative reactions. Vitamin C levels also declined over time since ascorbic acid is highly sensitive to oxygen, light, and temperature, and its decrease further supports the occurrence of oxidative reaction within the liquid core.

Consistent with these trends, antioxidant activity measured by both FRAP and ORAC assays showed a significant decrease during storage. The FRAP values dropped from 218.21 ± 7.22 μM Trolox/mL on Day 0 to 121.13 ± 2.14 μM Trolox/mL on Day 7, while ORAC values decreased from 1865.16 ± 5.42 to 1515.62 ± 37.05 μM Trolox/mL. These reductions reflect the loss of antioxidant compounds such as phenolics and vitamin C, which contribute directly to the overall antioxidant capacity of the LHBs.

To further improve the retention of bioactive compounds, future studies should focus on optimizing the encapsulation method, such as modifying membrane thickness, crosslinking density, or incorporating antioxidant-stabilizing agents. These improvements could enhance the protective function of the hydrogel matrix and extend the nutritional quality of LHBs during storage. Overall, these results suggest that cool storage and short-term consumption (within 3 days) are recommended to maximize nutritional quality.

3.7. Estimated glycemic index

LHB demonstrated a low estimated Glycemic Index (eGI) of 40. The Glycemic Index (GI) is a scale used to classify carbohydrate-containing foods based on how rapidly they elevate blood glucose levels post-consumption. A GI of 40 for the orange LHB indicates a slower rate of blood sugar increase, which can contribute to prolonged satiety. This characteristic makes the LHB particularly beneficial for elderly individuals, as it helps prevent rapid fluctuations in blood glucose levels.

3.8. Sensory evaluation and clinical trial

For the sensory evaluation of LHB designed for the elderly, a facial 5-point hedonic scale was utilized to assess various aspects of consumer preference, including color, aroma, taste, eating comfort, and overall acceptability. The scale ranged from 1 ("dislike very much") to 5 ("like very much"). The results of this evaluation are presented in Table 9. The color of the LHB received an average score of 3.97, indicating that 97 % of participants liked the color slightly, while 3 % did not enjoy it at all. The color, derived from freshly squeezed orange juice, is expected to appeal to potential consumers, who may rate it as "like slightly." In terms of aroma, 93 % of participants slightly liked the odor of the LHB, while 7 % neither liked nor disliked it, resulting in an average score of 3.93. This positive response suggests that the natural flavor from freshly pressed orange juice contributed significantly to the product's appeal.

The LHB' taste received an average score of 3.90. Of the 30 participants, 77 % slightly liked the taste, and 90 % found the product comfortable to consume, suggesting the texture and portion size were well-suited for elderly consumers. However, 10 % of participants were neutral on the taste, and 17 % expressed neutrality regarding the comfort of consumption. The appropriate serving size and small portion of the LHB made them a suitable option for the elderly.

Regarding overall acceptability, 77 % of participants slightly liked the orange LHB, while only 6 % slightly disliked them. Nevertheless, the findings indicate that the LHB was effective in alleviating dry mouth in the elderly while maintaining normal levels of hydration. Overall, 77 % of participants approved of the product across all evaluated characteristics: color, aroma, taste, eating comfort, and general acceptability.

In a 30-day clinical study involving 30 individuals, it was found that 60 % of subjects reported normal thirst levels, while 36.7 % experienced increased thirst, and only 3.3 % experienced a decrease in thirst (Fig. 3). This increased thirst may be attributed to the acidity of orange juice, which can cause a stronger sensation of thirst in elderly individuals. However, the study also showed that the LHB helped alleviate dry mouth in 66.7 % of participants. Additionally, 30 % of participants reported an increase in urination frequency after consuming the LHB, while the remaining 70 % experienced no changes. This increase in urination may be related to the additional water content provided by the LHB. Based on these findings, it can be concluded that the hydrogel not only helped alleviate dry mouth but also contributed to improved hydration in the elderly. This study has some limitations. First, only one LHB formulation was tested. Thus, future studies should compare different formulations to better understand how they affect sensory acceptance. Second, hydration status was evaluated using questionnaires, which do not directly reflect actual dehydration levels. Including objective hydration measurements would strengthen future research. Third, dietary intake, including the consumption of foods with high water content, was not recorded during the study period and may have influenced hydration outcomes. Finally, the quality of the orange juice was not controlled, which may affect the reproducibility of the results. Using standardized raw materials would help improve consistency in future studies.

Table 8
Total phenolic, vitamin C and antioxidant activity (FRAP, ORAC) of LHBs on different storage day.

Day	Total Phenolic (μg GAE /mL)	Vitamin C (mg/L)	Antioxidant activity (μM Trolox /mL)	
			FRAP Assay	ORAC Assay
0	130.99 ± 11.07[a]	50 - 100	218.21 ± 7.22[a]	1865.16 ± 5.42[a]
1	99.41 ± 2.47[b]	25 - 50	154.17 ± 6.25[b]	1753.85 ± 31.68[b]
3	92.27 ± 4.75[b]	25 - 50	159.22 ± 1.14[b]	1539.78 ± 24.71[c]
7	71.96 ± 1.12[c]	25 - 50	121.13 ± 2.14[c]	1515.62 ± 37.05[c]

Values are presented as mean ± SD (n = 3). Different superscript letters within a column indicate significant differences by Tukey's test ($p \leq 0.05$).

Table 9
Sensory acceptance of the LHB.

Parameter	Score (Mean ± SD)
Color	3.97 ± 0.18 (like slightly)
Aroma	3.93 ± 0.25 (like slightly)
Taste	3.90 ± 0.48 (like slightly)
Convenience	3.90 ± 0.31 (like slightly)
Overall	3.90 ± 0.48 (like slightly)

4. Conclusion

In this study, LHBs were developed using freshly squeezed orange juice as the liquid-core. The spherification technique was employed to form LHBs. Encapsulation of orange juice offers a practical approach to improving fluid intake, particularly for elderly individuals who may experience difficulties with conventional liquids. The results showed that LHBs maintained microbial safety for up to 7 days under refrigerated storage; however, changes in size, texture, and bioactive compound retention were observed over time. Notably, reductions in total phenolic

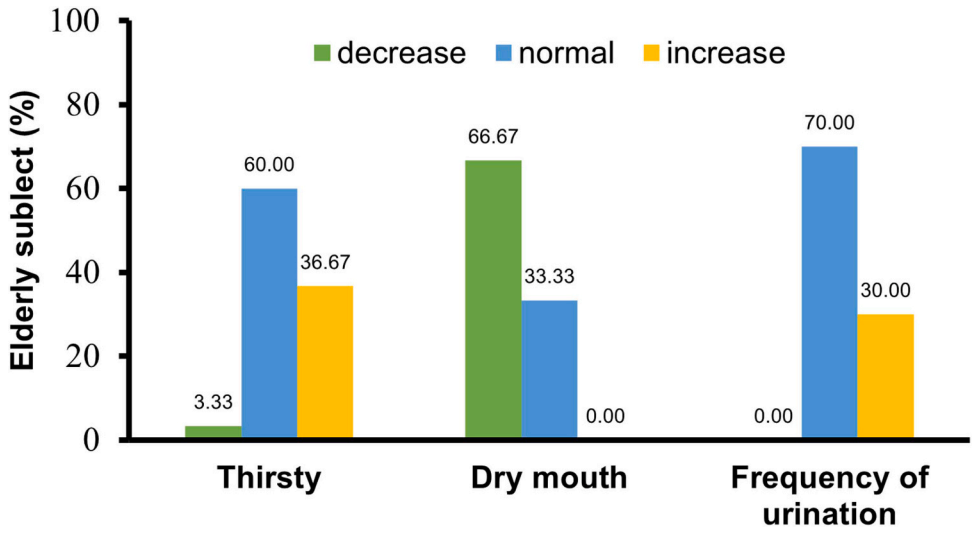

Fig. 3. Dehydration test in elderly.

content, vitamin C, and antioxidant activity indicated that the hydrogel membrane did not fully prevent oxidation and liquid migration during storage. Based on physical stability, texture homogeneity, and nutritional quality, consumption within a shorter storage period (approximately 3 days at 4°C) is recommended to maximize product quality. Sensory evaluation suggested that LHBs were acceptable and facilitate improved hydration. These findings not only present a promising strategy for improving hydration in the elderly but also emphasize the potential of using agricultural products in medical food development, paving the way for advancements in both nutrition and elderly care.

Ethical statement - studies in humans and animals

The study involved the participation of human subjects for sensory evaluation and was conducted in accordance with all applicable laws and institutional guidelines. Informed consent was obtained from all participants prior to their involvement in the research. Throughout the study, the privacy rights of all individuals were fully respected and upheld.

CRediT authorship contribution statement

Wimonphan Chathiran: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Worakrit Saiyasombat:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Janejira Wongpiyapun:** Writing – original draft, Investigation, Formal analysis, Data curation. **Jaruwan Chimasangkanan:** Methodology, Formal analysis. **Pimpinan Somsong:** Funding acquisition, Conceptualization. **Keshavan Niranjan:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **Warangkana Srichamnong:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101730.

Data availability

Data will be made available on request.

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