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Original Article

Utilization of glucomannan-xanthan gum-carrageenan based co-processed excipients in the development of nutraceutical pomegranate (*Punica granatum* L.) peel extract jelly

[Utilización de excipientes coprocesados a base de glucomanano-goma xantana-carragenina en el desarrollo de gelatina nutracéutica de extracto de cáscara de granada (*Punica granatum* L.)]

Ronald Horison, Silvia Surini*

Laboratory of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, 16424, Indonesia.
*E-mail: silvia@farmasi.ui.ac.id

Abstract

Context: The expanding product market presents an opportunity for the creation of innovative nutraceuticals incorporating pomegranate (*Punica granatum* L.) peel extract (PPE) in attractive dosage forms. In this context, novel dosage forms such as jelly are widely accepted due to their appealing qualities. However, achieving a desirable chewy texture throughout jelly production necessitates the use of a gelling agent with high gel strength, prompting the exploration of superior excipients. This study focuses on the development and application of new excipient combinations, namely carrageenan, xanthan gum, and glucomannan, as gelling agents in pomegranate peel extract jelly (PPEJ).

Aims: To develop new co-processed excipients consisting of glucomannan, xanthan gum, and carrageenan and assess the suitability of developing PPEJ as a nutraceutical product.

Methods: A total of eight formulae of co-processed excipients were obtained through the co-drying process and were physically, chemically, and functionally characterized. The developed excipients were used as gelling agents in nutraceutical PPEJ and subjected to physical and hedonic evaluation.

Results: Co-processing improved excipient's functional qualities, while negligible chemical interactions were verified by FT-IR, DSC, and XRD analyses. Furthermore, GLU-XAG-CAR-7 had the highest gel strength value (1659.25 ± 70.17 g) and was selected for jelly formulation. Texture and hedonic evaluations showed that PPEJ-C had the highest GLU-XAG-CAR-7 concentration formula and produced the best jelly.

Conclusions: The new co-processed excipients show remarkable potential to be used as a gelling agent in the formulation of PPEJ.

Keywords: co-processed excipients; hydrocolloids; jelly; nutraceutical; pomegranate peel extract.

Resumen

Contexto: El mercado de productos en expansión presenta una oportunidad para la creación de nutracéuticos innovadores que incorporen extracto de cáscara de granada (*Punica granatum* L.) (PPE) en atractivas formas de dosificación. En este contexto, las nuevas formas de dosificación como la gelatina son ampliamente aceptadas debido a sus atractivas cualidades. Sin embargo, lograr una textura masticable deseable durante toda la producción de gelatina requiere el uso de un agente gelificante con alta fuerza de gel, lo que impulsa la exploración de excipientes superiores. Este estudio se centra en el desarrollo y la aplicación de nuevas combinaciones de excipientes, a saber, carragenina, goma xantana y glucomanano, como agentes gelificantes en la gelatina de extracto de cáscara de granada (PPEJ).

Objetivos: Desarrollar nuevos excipientes coprocesados que consistan en glucomanano, goma xantana y carragenina y evaluar la idoneidad de desarrollar PPEJ como un producto nutracéutico.

Métodos: Se obtuvieron un total de ocho fórmulas de excipientes coprocesados mediante el proceso de cosecado y se caracterizaron física, química y funcionalmente. Los excipientes desarrollados se utilizaron como agentes gelificantes en PPEJ nutracéutico y se sometieron a evaluación física y hedónica.

Resultados: El co-procesamiento mejoró las cualidades funcionales del excipiente, mientras que las interacciones químicas insignificantes se verificaron mediante análisis FT-IR, DSC y XRD. Además, GLU-XAG-CAR-7 tuvo el valor de fuerza de gel más alto (1659,25 ± 70,17 g) y fue seleccionado para la formulación de gelatina. Las evaluaciones de textura y hedónicas mostraron que PPEJ-C tuvo la fórmula de concentración más alta de GLU-XAG-CAR-7 y produjo la mejor gelatina.

Conclusiones: Los nuevos excipientes coprocesados muestran un potencial notable para ser utilizados como agente gelificante en la formulación de PPEJ.

Palabras Clave: excipientes coprocesados; extracto de cáscara de granada; hidrocoloides; gelatina; nutracéutico.

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AUTHOR INFO
ORCID: 0000-0002-6523-3185 (RH) 0000-0003-1211-9706 (SS)

INTRODUCTION

The increase in consumption of nutraceutical products is showing an important role in sustaining optimal functional health and mitigating the onset of chronic diseases (Egbuna and Dable-Tupas, 2020; Puri et al., 2022). This trend fuels the potential of developing novel nutraceutical products. The bioactive component with the potential to be used as a nutraceutical product is pomegranate (*Punica granatum* L.) peel extract (PPE), which is rich in polyphenols and tannins. PPE shows antioxidant, anti-inflammatory, antibacterial, antiviral, and preventive effects against cancer, diabetes, hypercholesterolemia, and cardiovascular disease (Mo et al., 2022; Pirzadeh et al., 2021). In addition, existing PPE health supplements are available in conventional pharmaceutical dosage forms such as powders, tablets, and capsules, opening opportunities for development into appealing dosage forms.

An innovative pharmaceutical dosage form developed is jelly, which possesses a chewy texture, unique appearance, and sweet taste to be more palatable by consumers from diverse age groups (Grigio et al., 2022; Zokaityte et al., 2021). The incorporation of pomegranate peel extract in the jelly formulation allows the extract to be presented to the consumer in a more attractive way due to its unique shape, appealing color, and chewy texture. These features provide an impression of consuming the product as a snack instead of a drug to improve patient compliance. The development of jelly uses hydrocolloid gel-forming excipients to form a three-dimensional network, requiring excipients with high gel strength. Therefore, developing novel excipients with superior characteristics is essential in jelly development.

Co-processed excipients offer a promising approach to fulfilling the required excipient properties in jelly formulation. The combination of glucomannan, xanthan gum, and carrageenan (GLU-XAG-CAR) shows synergistic interactions in gel formation through the improvement of strength and swelling capabilities (Ghebremedhin et al., 2020; Tunieva et al., 2021; Wu et al., 2020). In this study, a co-drying method was used to prepare GLU-XAG-CAR co-processed excipients, as a gelling agent for pomegranate peel extract jelly (PPEJ). Therefore, this study was done to help understand the impact of excipient compositions on GLU-XAG-CAR properties and its potential in the formulation of PPEJ as an attractive nutraceutical product.

MATERIAL AND METHODS

Chemicals and reagents

Food-grade glucomannan (96.3%) (Shanxi Pioneer Biotech, China), pharmaceutical-grade xanthan gum (95.3%) (Grinsted Xanthan 80 PRM Plus (Danisco, Denmark), and pharmaceutical-grade carrageenan (Gelcarin GP911 NF) (Dupont, United States) were selected as materials for the co-processed excipients. Meanwhile, PPE (Wellgreen Technology, China) and ellagic acid (Hunan Insen Biotech, China) were purchased as active ingredients. The additional excipients provided were stevia sweetener (Nutrifood, Indonesia), ascorbic acid (CSPC Weisheng Pharmaceutical, China), food-grade sodium benzoate, citric acid, vanillin, and grape flavorings (Gunacipta Multirasa, Indonesia). Furthermore, acetonitrile and methanol at liquid chromatography grade (Merck) with high purity (>99%) were used as solvents.

Preparation of GLU-XAG-CAR excipients

Glucomannan, xanthan gum, and carrageenan were co-processed using a co-drying method (Surini et al., 2018). Individual excipient dispersions in distilled water were prepared as specified in Table 1 before blending. The mixture was homogenized for 30 minutes at 800 rpm using a homogenizer (IKA, Germany). Subsequently, the homogenized mixtures were co-dried in a drum dryer (Simmons, United Kingdom) at 110 ± 5°C and 1.5 rpm. The resulting flakes were pulverized into powder, which passed a 25-mesh sieve, and the fine powder was subjected to a drying process using an oven dryer at 40°C for 6 h (Al-Akayleh et al., 2013).

Characterization of GLU-XAG-CAR excipients

Moisture content

Determination of the moisture content of GLU-XAG-CAR gravimetrically was conducted using a moisture analyzer (VWR, United States) for approximately ± 1 gram powder through heating at 105°C for 5 min (Vodáčková et al., 2018).

FT-IR spectroscopy

A ThermoScientific Nicolet IS50 Infrared spectrophotometer (FT-IR) (ThermoScientific, United States) was used to confirm and detect the impact of co-processing towards chemical interactions among components of excipients. The evaluation was conducted at wavelengths of 4000–400 cm^{-1} with a sensitivity value, resolution, and threshold of 50, 2 cm^{-1},

Table 1. Composition of GLU-XAG-CAR excipients.

Formula	Concentration (% w/w)		
	Glucomannan	Xanthan gum	Carrageenan
GLU-XAG-CAR-1	3	3	3
GLU-XAG-CAR-2	3	3	5
GLU-XAG-CAR-3	3	5	3
GLU-XAG-CAR-4	3	5	5
GLU-XAG-CAR-5	5	3	3
GLU-XAG-CAR-6	5	3	5
GLU-XAG-CAR-7	5	5	3
GLU-XAG-CAR-8	5	5	5

and 1.303, respectively. Meanwhile, the IR spectra of GLU-XAG-CAR were compared with the individual excipients (Jade et al., 2017).

Thermal properties

The thermal properties study used Differential Scanning Calorimetry (DSC) (PerkinElmer, United States), which was carried out for 1.5 mg powder. The procedure was conducted in an aluminum pan at a linear heating rate of 5°C/min for a temperature range of 30–350°C with 30 mL/min of nitrogen as purging gas (Eraga et al., 2017; Jade et al., 2017).

X-ray diffraction

Evaluation of excipients crystallinity was performed using an X-ray Diffractometer (XRD) (Shimadzu, Japan) at 2θ conditions ranging from 5° to 80°. Observation of peak shifting of GLU-XAG-CAR diffractogram patterns is compared to the parent's excipients (Ghumman et al., 2019; Jade et al., 2017).

Surface morphology

The morphological surface characteristics of the powder were observed with a Scanning Electron Microscope (SEM) (JEOL, Japan). The powder was fixed on an aluminum stub and sputter-coated with gold. Furthermore, the samples were observed at an acceleration voltage of 10 kV for 2500× magnifications (Svačinová et al., 2019).

Particle diameter

The particle size distribution of GLU-XAG-CAR powder was conducted using a sieve shaker (Retsch, Germany) through a sieve at sizes 35, 45, 60, 80, 120, and 230 mesh. Approximately 50 g of excipient powder, which vibrated at 0.4 amplitude for 20 minutes, was tested. The weight distribution was determined by the mass of excipients at each sieve. The normal distribution method calculated the statistical diameter based on the weight data (Ryckaert et al., 2021).

Flow properties

The flow property of excipient powder was evaluated by determining flow speed, angle of repose, Hausner's Ratio (HR), and Compressibility Index (CI). The flowability of co-processed excipients was determined using a testing device (Erweka, Germany), where approximately 20 g of excipients were placed in the flow device. The time consumed for excipients to flow through the orifice was recorded. Determining the angle of repose was carried out through the fixed funnel method calculated as the inverse of a tangent from the cone measured according to [1]. In this equation, AR is the angle of repose, h is the height of the sample cone, and r is the radius of the base of the cone (Rathod et al., 2019).

$$AR = \tan^{-1}\left(\frac{h}{r}\right) \quad [1]$$

HR and CI excipient flow was affected by particle intra-particulate interaction. The volume of ±5 g excipient powder without tapping was reported as bulk volume used to determine the bulk density (BD). Meanwhile, tapped density (TD) was calculated as the ratio of excipient mass with volume occupied by the sample after tapping for 300 taps. HR was calculated as the ratio of tapped to BD, while CI was obtained according to [2] (Rathod et al., 2019).

$$CI\ (\%) = \frac{TD - BD}{TD} \times 100\% \quad [2]$$

Swelling capability

The swelling index of co-processed excipients was determined through a ratio of improved volume at a glass cylinder after submersion into distilled water.

Table 2. Formulation of pomegranate peel extract jelly (PPEJ).

Material	Concentration (% w/w)		
	PPEJ-A	PPEJ-B	PPEJ-C
GLU-XAG-CAR	3	4	5
PPE	1	1	1
Stevia sweetener	3	3	3
Sodium benzoate	0.10	0.10	0.10
Ascorbic acid	0.02	0.02	0.02
Vanillin	0.02	0.02	0.02
Grape flavorings	0.20	0.20	0.20
Citric acid	0.02	0.02	0.02
Distilled water	Ad 100	Ad 100	Ad 100

GLU: glucomannan; XAG: xanthan gum; CAR: carrageenan; PEE: pomegranate peel extract.

The calculation of the swelling index (SI) was carried out following [3] with excipient volume before (V_0) and after submerging for a particular time (V_t) (Assaf et al., 2019).

$$\text{SI}\ (\%) = \frac{V_t - V_0}{V_0} \times\ 100\% \quad [3]$$

Dispersion viscosity

Excipient viscosity and rheology profile were investigated through analysis of GLU-XAG-CAR 2% (w/w) dispersion using a rotational viscometer (ColePalmer, United States) (Surini et al., 2018).

Dispersion gel strength

GLU-XAG-CAR 3% (w/w) excipient dispersion gel strength was assessed using a texture analyzer (Perten, United States) through a single compression method. The testing was carried out using a probe with a cylinder probe with 5 mm diameter and 40 mm length with 10 mm/s initial speed, 1 mm/s test, and retract speed (Fateha et al., 2021; Günter et al., 2022).

Formulation of PPEJ

PPEJ was prepared with materials listed in the formulations as presented in Table 2. Dry ingredients, excluding the extract and GLU-XAG-CAR, were dissolved in distilled water. Subsequently, the extract powder was blended into the mixture, and GLU-XAG-CAR was incorporated into the mixture, which was maintained at 80°C for 2 min. The resulting mixture was deposited into jelly molds and refrigerated at 7 ± 3°C for 30 min. Before further analysis, the jellies were stored in the refrigerator (Cano-Lamadrid et al., 2020; Enache et al., 2021; Grigio et al., 2022).

Characterization of jelly containing PPEJ

Texture profile analysis (TPA)

TPA of jellies for each formula was performed using a texture analyzer (Stable Microsystems, United Kingdom). A double-compression cycle method was performed with pre-test, test, and post-test speeds of 1 mm/s, 2 mm/s, and 10 mm/s respectively. The obtained force-time graphs were analyzed for hardness, springiness, cohesiveness, and chewiness (Delgado and Bañón, 2015; Renaldi et al., 2022).

Ellagic acid content

Ellagic acid content was analyzed using an HPLC instrument (Shimadzu, Japan) with a UV detector set at 254 nm and a C-18 column. Elution used a gradient mobile phase composed of acetonitrile (solvent A) and distilled water (solvent B) at a 0.5 mL/min flow rate. Furthermore, the gradient program started with 50% min, 2–4 min, 4–6 min, 6–8 min, and 8–10 min with 50% A, 50–60% A, 60% A, 60–65% A, and 65–40% A, respectively (Jingjing and Qipeng, 2008; Wu et al., 2021).

Hedonic study

This hedonic study was conducted in accordance with the ethical principles of the Declaration of Helsinki. All methods used in this evaluation were carried out in accordance with the ethical standards and regulations for sensory evaluation, approved by the Health Research Ethics Committee–Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo Hospital (HREC-FMUI/CMH) (Approval no: KET-1418/UN2.F1/ETIK/PPM.00.02/2023). A total of thirty untrained panelists evaluated the PPEJ sample's appearance, taste, aroma, chewability, and over-

all likeness using a seven-point hedonic scale (7: highly liked, 1: highly disliked) (Čižauskaite et al., 2019; Grigio et al., 2022).

The evaluation panelists were healthy adults (18–54 years old) who voluntarily agreed to participate in the evaluation testing, proven by signing the informed consent form. The panelist exclusion criteria are pregnant and lactating women, smokers, and subjects with an allergy history to the ingredients used in the jelly formula.

Dissolution study

The dissolution profile of PPEJ was investigated using a tester (Electrolab, India) under the type 2 method at 37°C with distilled water as the medium (Karaiskou et al., 2020). The testing used intact and squashed samples for each formula to mimic the chewing process during consumption.

Stability study

PPEJ samples were subjected to stability evaluations for 24 weeks at 30°C and 5°C while stored in a glass container with a moisture absorber. Samples were taken out for weight and ellagic acid level examination at predetermined intervals (Elshafeey and El-Dahmy, 2022; Vojvodić-Cebin et al., 2024).

Statistical analysis

The results were presented as mean accompanied with ± standard deviation. Statistical analysis of one-way ANOVA at SPSS-23 was performed to determine the significant differences at probability value ($p<0.05$). The experiments for each test were done in three replicates ($n = 3$).

RESULTS AND DISCUSSION

Development of GLU-XAG-CAR co-processed excipients

This study used co-drying to facilitate the drying process for the mixed viscous slurry dispersion to create GLU-XAG-CAR excipients (Domínguez, 2011). The drying process produced a fine white-yellow powder with no significant differences from the appearance of any GLU-XAG-CAR formulation. GLU-XAG-CAR's production yield ranged between 51.8% to 83.5% due to weight loss during the manufacturing processes.

GLU-XAG-CAR powder characteristics

Powder flow and flowability behavior significantly affected the manufacturing process efficiency and product quality attributes. These characteristics of developed excipients were examined, while the flow properties of GLU-XAG-CAR were evaluated by flow rate, angle of repose, CI, and HR, as reported in Table 3. The flow rate of GLU-XAG-CAR ranged from 3.70 ± 0.10 g/s to 19.73 ± 0.46 g/s, with GLU-XAG-CAR-3 presenting the highest. The angle of repose was a relative measurement of friction and cohesion among particles influenced by particle size. For GLU-XAG-CAR, the angle of repose varied from 20.30 ± 1.21° to 30.93 ± 0.53°, which was considered excellent except for GLU-XAG-CAR-2 and GLU-XAG-CAR-6 reported to have a good flow. The developed excipient's moisture content varied from 10.470 ± 0.038% to 10.580 ± 0.035%, affecting the powder flowability.

The results showed the tendency of the role of parent excipient composition in determining the flow properties of developed GLU-XAG-CAR. Serrano-Mora et al. (2021) reported that particle size and morphology acted as antecedent factors in the flow rate of powder material. Therefore, powder with uniform shape and size possessed prominent flow behavior (Ćirin-Varađan et al., 2022). The interparticle interactions analyzed from CI and HR value affected the flowability. All GLU-XAG-CAR formulae possess HR values higher than 1.45 and are accompanied by CI ranging from 32–37%. Poor flowing properties caused by high friction due to intraparticular interaction can also be concluded from the significant difference between bulk and TD properties (Rojas and Kumar, 2012).

Table 3 shows the particle diameter of GLU-XAG-CAR, where most GLU-XAG-CAR formulae have a diameter size ranging from 506.4170 µm to 559.3223 µm. Powder flow properties are directly impacted by particle size and shape. Due to the smaller surface area, larger particles typically have a higher flowability rate, causing a decrease in the adhesiveness of the materials. Narrow size distribution prevents segregation and agglomeration problems during the blending process. Therefore, co-processed excipients with narrow particle size distribution have better flow properties compared to a single form or a physical mixture, as reported by Ćirin-Varađan et al. (2022).

Surface morphology observation

The surface morphology of excipients was examined using SEM, as reported in Fig. 1. Topographical features of excipient powder surface essentially contribute towards inadmissible flow properties as the formed particles clump by interacting with each other's surfaces (Patil et al., 2021). Compared with parent excipients, the developed GLU-XAG-CAR shows significant differences in shape and topography. Several quantities of tiny particle components were adsorbed on the surface of co-processed particles.

Table 3. Particle and flow properties of GLU-XAG-CAR.

Formula	Angle of repose (°)	Flow rate (g/s)	Density (g/mL)		CI (%)	HR	Moisture content (%)	Particle diameter (µm)
			Tapped	Bulk				
GLU-XAG-CAR-1	28.06 ± 1.60^{bc}	7.00 ± 0.36^{b}	0.093 ± 0.002^{c}	0.145 ± 0.002^{c}	35.80 ± 0.59^{c}	1.56 ± 0.01^{abc}	10.580 ± 0.035^{b}	521.7742 ± 1.1427^{a}
GLU-XAG-CAR-2	30.55 ± 1.40^{cd}	3.70 ± 0.10^{a}	0.054 ± 0.001^{a}	0.085 ± 0.001^{a}	36.10 ± 0.56^{abc}	1.57 ± 0.01^{abc}	10.513 ± 0.029^{ab}	546.4322 ± 1.2682^{d}
GLU-XAG-CAR-3	26.34 ± 0.76^{b}	13.03 ± 0.46^{d}	0.104 ± 0.001^{de}	0.165 ± 0.004^{de}	49.24 ± 2.17^{de}	1.97 ± 0.05^{c}	10.462 ± 0.049^{ab}	526.4945 ± 1.6060^{b}
GLU-XAG-CAR-4	25.85 ± 0.36^{b}	6.50 ± 0.40^{b}	0.077 ± 0.003^{b}	0.126 ± 0.007^{b}	38.91 ± 0.73^{b}	1.64 ± 0.02^{b}	10.549 ± 0.013^{ab}	524.2612 ± 0.5802^{ab}
GLU-XAG-CAR-5	20.30 ± 1.21^{a}	19.73 ± 0.46^{e}	0.108 ± 0.002^{e}	0.174 ± 0.001^{e}	37.85 ± 0.77^{e}	1.61 ± 0.02^{b}	10.497 ± 0.024^{ab}	534.5022 ± 1.1566^{c}
GLU-XAG-CAR-6	30.93 ± 0.53^{d}	3.97 ± 0.29^{a}	0.079 ± 0.001^{b}	0.120 ± 0.001^{b}	34.36 ± 1.03^{b}	1.52 ± 0.02^{a}	10.448 ± 0.048^{ab}	556.1760 ± 1.3402^{e}
GLU-XAG-CAR-7	27.87 ± 0.30^{bc}	7.13 ± 0.25^{b}	0.092 ± 0.003^{c}	0.139 ± 0.004^{c}	34.15 ± 0.35^{c}	1.52 ± 0.01^{a}	10.407 ± 0.038^{a}	559.1656 ± 1.0943^{f}
GLU-XAG-CAR-8	27.82 ± 0.60^{b}	9.23 ± 0.23^{c}	0.099 ± 0.004^{d}	0.155 ± 0.006^{d}	36.16 ± 1.93^{d}	1.57 ± 0.05^{ab}	10.426 ± 0.010^{ab}	557.2284 ± 0.6693^{ef}

Moisture content, density values, angle of repose, flow rate, compressibility index, Hausner's ratio, and particle diameter are expressed as mean ± SD (n = 3). Data followed with different letters are significantly different by ANOVA followed by Tukey's test ($p<0.05$). GLU: glucomannan; XAG: xanthan gum; CAR: carrageenan; CI: Compressibility index; HR: Hausner's ratio (HR).

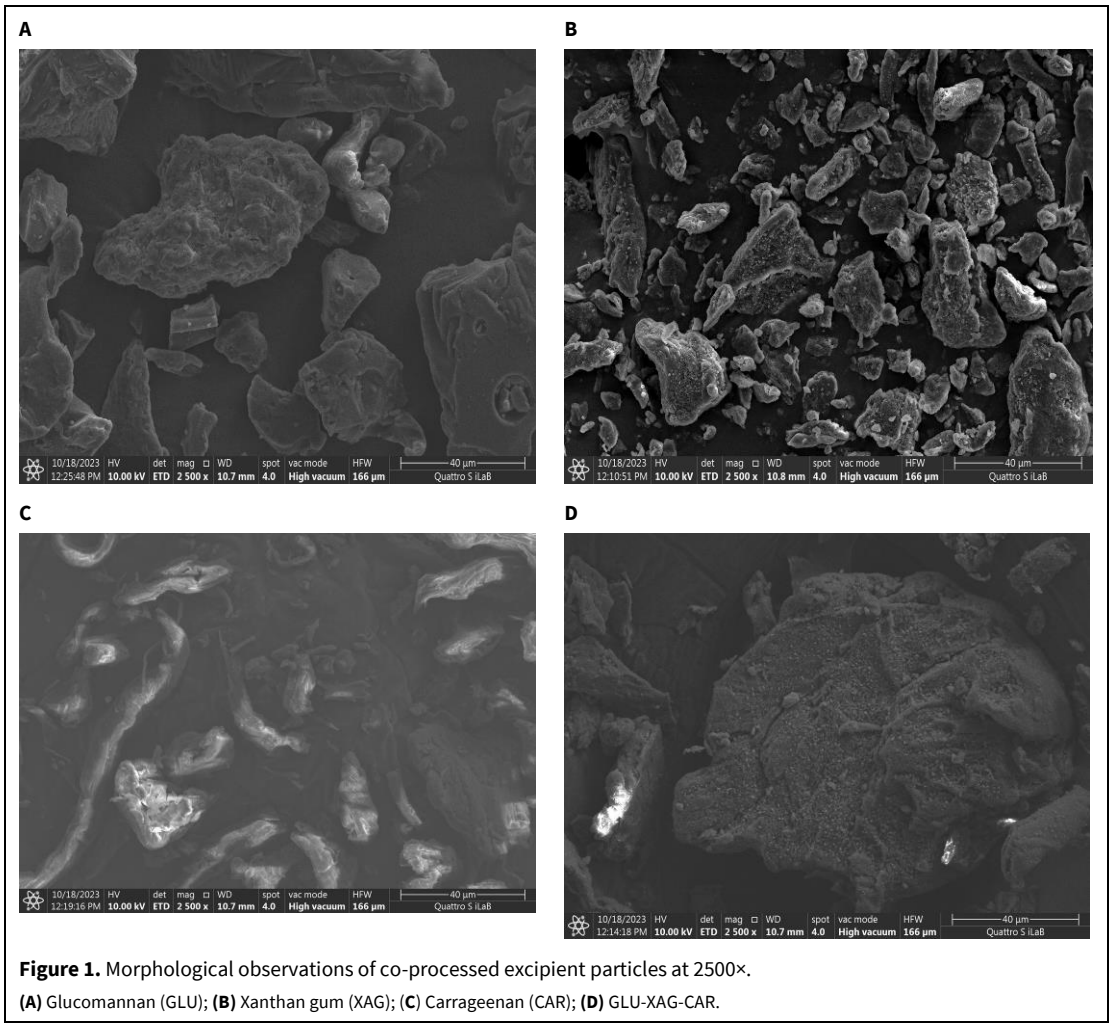

Figure 1. Morphological observations of co-processed excipient particles at 2500×.
(A) Glucomannan (GLU); **(B)** Xanthan gum (XAG); (**C**) Carrageenan (CAR); **(D)** GLU-XAG-CAR.

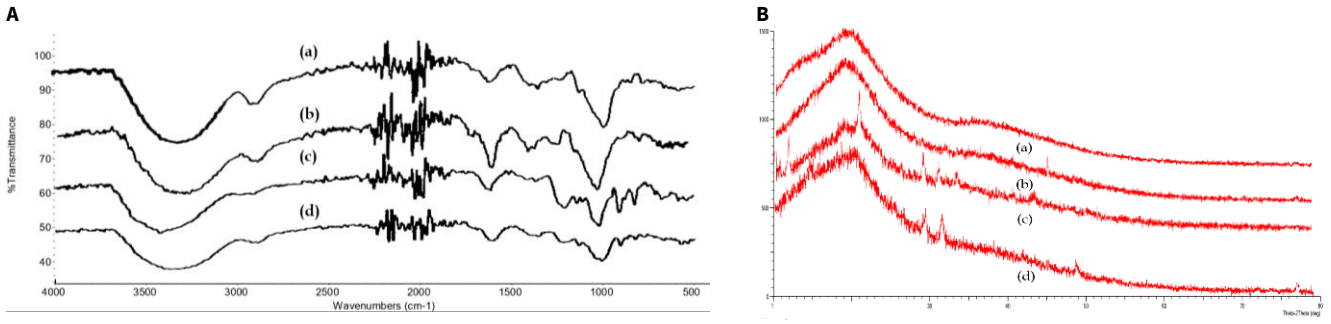

Figure 2. IR spectra **(A)** and crystallinity diffractogram **(B)** of excipients.
(a) Glucomannan (GLU); **(b)** Xanthan gum (XAG); (c) Carrageenan (CAR); **(d)** GLU-XAG-CAR.

Glucomannan, xanthan gum, and carrageenan were all indistinguishable at the co-processed excipients photomicrograph, showing that the materials were homogeneously blended as a single constituent.

Functional groups analysis

The FT-IR analysis results in the spectrogram are presented in Fig. 2. The GLU-XAG-CAR spectra were observed to have various peaks similar to the parent excipient spectra. Intensity weakening phenomena at GLU-XAG-CAR have been at 1015.93 cm^{-1}, corresponding to the vibration of the C=O bond. The spectra showed other bands, while a peak at 1601 cm^{-1} corresponding to C-O at enol functional groups from xanthan gum reported the same phenomenon at GLU-XAG-CAR spectra. The phenomenon of peak shifting, attributed to the sulphate functional group, was observed in the GLU-XAG-CAR spectra, transitioning from 842.05 cm^{-1} to 928.92 cm^{-1}. The appearance and dismission of new and old bands were not observed at GLU-XAG-CAR spectra, which confirmed the absence of any detectable chemical interaction. The study showed no chemical changes due to the co-processing following similar previous results (Benabbas et al., 2021).

Crystallinity properties study

The X-ray diffractograms of parent excipients and GLU-XAG-CAR showed a semi-crystalline structure. Glucomannan, xanthan gum, carrageenan, and GLU-XAG-CAR report crystallinity rates of 10.3336%, 15.4645%, 23.1440%, and 16.7693%, respectively. Broad diffraction peaks established the amorphous property of the xanthan gum, ranging from 17.9380°-21.2455° due to double helix conformation (Rao et al., 2010). Furthermore, the GLU-XAG-CAR diffractogram showed sharp peaks at 21.5668°, 29.6284°, and 31.8333°, confirming the presence of carrageenan lattice with similar peaks at 20.7919°, 29.1778° and 31.1656°, respectively. GLU-XAG-CAR reported slight shifting and intensity reduction of carrageenan diffraction peaks, confirming the formation of a composite blend due to physical interaction through co-processing (Patil et al., 2021). Water-induced structural transitions associated with amorphous-crystalline from the natural interactions between polysaccharides and water may account for the phenomena observed during co-processing (Trisopon et al., 2020).

Thermal properties study

The obtained thermogram corresponded to glucomannan, xanthan gum, carrageenan, and GLU-XAG-CAR exhibited different thermal behaviors. The existence of an endothermic peak that represents glass transition temperature was observed at glucomannan (60.75°C), xanthan gum (65.76°C), and GLU-XAG-CAR (68.88°C) thermogram (Fig. 3). Additional endotherm peaks were also observed at the glucomannan thermogram (226.88°C), representing the transformation of thermoplastic gel into extrudable melt (Leuangsukrerk et al., 2014). Meanwhile, the exothermic peaks are detected at the xanthan gum (275.28°C) and carrageenan (211.71°C) thermograms corresponding to the melting points. The thermogram of GLU-XAG-CAR showed a combination of the thermal phenomena with a peak shifting in the glass transition and melting temperature compared to the characteristics of the materials. The thermogram result showed that there was no significant chemical interaction in developed co-processed excipients. The physical interaction due to the co-processing was observed with shifting of exotherm and endotherm peak at GLU-XAG-CAR thermogram.

GLU-XAG-CAR dispersion characteristics

Functional characteristics of GLU-XAG-CAR excipient dispersions are shown in Table 4. Gel strength is defined as resistance towards external pressure correlated to the cohesiveness attribute of materials. A strong gel network is reported by a higher strength value, and the highest is observed at GLU-XAG-CAR-7 dispersion. Improvement of gel strength at

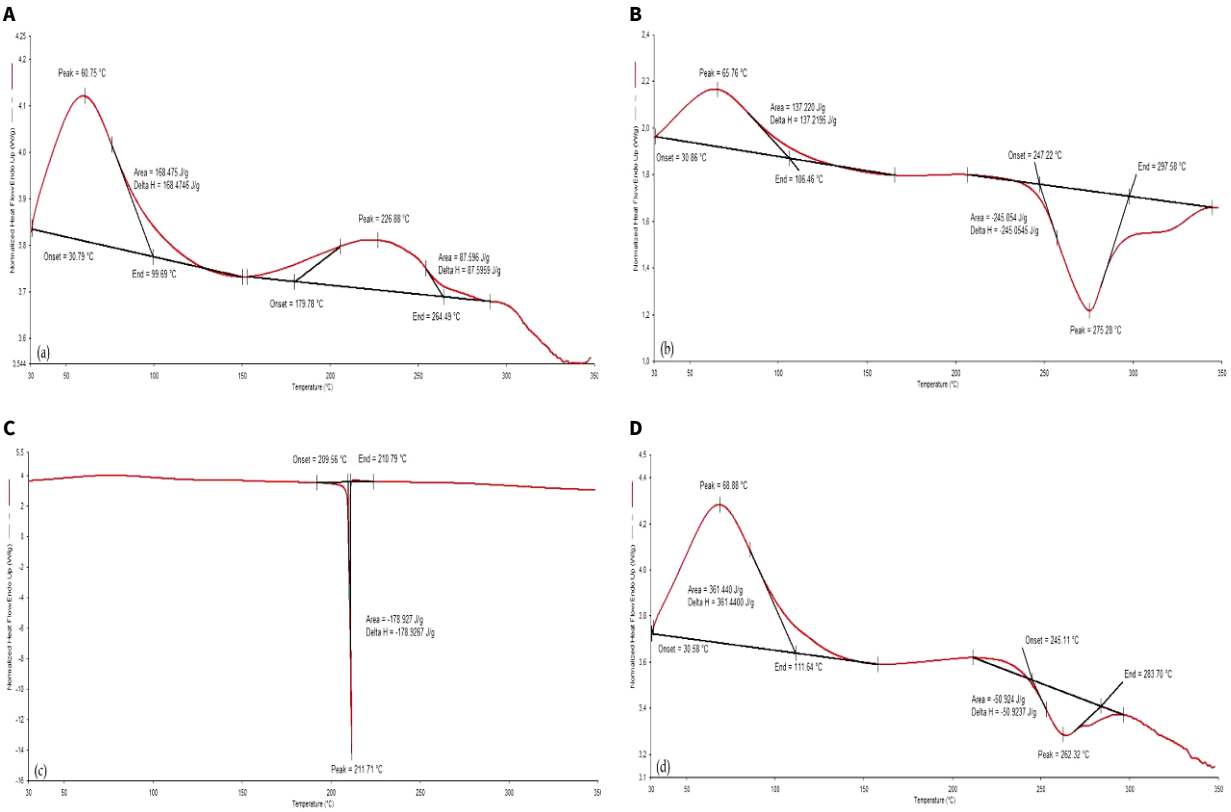

Figure 3. Endothermic and exothermic curves of excipients.

(A) Glucomannan (GLU); **(B)** Xanthan gum (XAG); **(C)** Carrageenan (CAR); and **(D)** GLU-XAG-CAR. GLU: glucomannan; XAG: xanthan gum; CAG: carrageenan.

Table 4. Physicochemical characteristics of GLU-XAG-CAR dispersions.

Formula	pH	Gel strength (g)	Swelling index (%)	Viscosity (Cp)
GLU-XAG-CAR-1	7.48 ± 0.01^{c}	1437.25 ± 46.31^{c}	353.3 ± 11.5^{bcd}	4266.37 ± 2.55^{e}
GLU-XAG-CAR-2	7.82 ± 0.01^{e}	893.25 ± 47.49^{a}	209.5 ± 21.8^{a}	7537.03 ± 7.78^{h}
GLU-XAG-CAR-3	7.28 ± 0.01^{a}	916.50 ± 48.83^{a}	580.0 ± 34.6^{e}	2610.97 ± 0.87^{b}
GLU-XAG-CAR-4	7.93 ± 0.01^{f}	1202.75 ± 102.41^{b}	433.3 ± 33.3^{d}	2592.60 ± 2.21^{a}
GLU-XAG-CAR-5	7.27 ± 0.01^{a}	1579.25 ± 87.58^{cd}	440.0 ± 40.0^{d}	4057.40 ± 1.65^{c}
GLU-XAG-CAR-6	7.54 ± 0.01^{d}	1598.25 ± 60.95^{d}	286.7 ± 11.5^{ab}	4223.27 ± 1.36^{d}
GLU-XAG-CAR-7	7.28 ± 0.01^{a}	1659.25 ± 70.17^{d}	327.8 ± 41.9^{bc}	4490.37 ± 1.31^{f}
GLU-XAG-CAR-8	7.36 ± 0.01^{b}	1437.25 ± 46.31^{c}	380.0 ± 20.0^{cd}	6742.70 ± 6.35^{g}

Moisture content, pH, gel strength, swelling index, and viscosity are expressed as mean ± SD (n = 3). Data followed with different letters are significantly different by ANOVA followed by Tukey's test at a 5% level of significance ($p<0.05$). GLU: glucomannan; XAG: xanthan gum; CAR: carrageenan.

co-processed excipients is achieved due to the entanglement of the polymer chain at co-processing, which facilitates the interpenetrating polymer network.

The swelling index can be described as an improvement of excipient volume, showing the ability to absorb water. The data in Table 4 varied from 209.5 ± 21.8% to 580.0 ± 34.6%, and the ability of excipients to interact with water was correlated with the dispersions' capacity to swell. Similar to gel strength, swelling index values were affected by excipient composition.

Table 4 presents the viscosity of the GLU-XAG-CAR dispersion, which differs based on composition and ranges from 2592.60 ± 2.21 Cp to 7537.03 ± 7.78 Cp. All GLU-XAG-CAR formulae showed shear thinning or pseudoplastic features in the rheogram shape

and a decline in viscosity when the shear rate increased. This behavior was similar to the parent excipients, where co-processing did not affect the changes observed (Sangnim et al., 2021).

Development of PPEJ

PPEJ was formulated using GLU-XAG-CAR-7, as shown by the highest gel strength result, which correlated to the chewiness and cohesiveness of the jelly. The formulation was brown, gummy bear-shaped, and possessed the required cohesiveness. There was no significant difference in the appearance and textural aspect of each formula. Each PPEJ weighted ± 850 mg without any significant differences, which contained 3.83 ± 0.09, 4.15 ± 0.22, and 4.16 ± 0.20 mg of ellagic acid for each unit of PPEJ-A, PPEJ-B, and PPEJ-C without significant difference as presented at Table 5.

Texture profile

Texture plays an important role in determining the product's physical quality. Profile analysis of PPEJ was carried out to evaluate the texture characteristics by observing changes during the compression process. The textural properties were reported based on various parameters such as hardness, springiness, cohesiveness, chewiness, and resilience, as shown in Table 6. Hardness shows the required force for a sample to be subjected to structural deformation. Springiness is defined as the ability of the sample to return to its initial condition after the deformed pressure is removed, which shows elasticity. Meanwhile, cohesiveness is related to the internal strength of the product and the difficulty in breaking the bonds. Chewiness corresponds to the force or work required to masticate the sample in the oral cavity (Elshafeey and El-Dahmy, 2022). In this context, PPEJ hardness is significantly dependent on GLU-XAG-CAR concentration. Cohesiveness, springiness, and chewiness of PPEJ were not significantly different in increasing GLU-XAG-CAR concentration. Mohammadi-Moghaddam et al. (2020) reported that the improvement of gelatin as a gelling agent significantly improved the hardness, cohesivity, elasticity, and chewiness of Masghati, Iranian jelly-like sweets.

In vitro drug release study

The concentration of GLU-XAG-CAR and the squashing treatment influence the cumulative release of ellagic acid in PPEJ, as shown in Fig. 4. Furthermore, the percentage of ellagic acid dissolved after 2 h ranges from 65.70 ± 2.98% to 94.33 ± 2.36%. The dissolution of active pharmaceutical ingredients from jelly is caused by water diffusion, polymer chain relaxation, jelly erosion, and swelling. Due to the polymer matrix's high retardancy, drug release was reduced at higher gelling agent concentrations. These results are consistent with Zhou et al. (2022), where concentrations of gelatine ranging from 6% to 12% had an impact on the amount of vitamin C released during the development of gelatine-based gummy jelly full-stop. Elshafeey and El-Dahmy (2022) reported a similar phenomenon that gelling agent type and concentration had a significant impact on the amount of dissolved etilefrine hydrochloride on oral medicated jelly compared to intact jelly, the smaller PPEJ sizes obtained from the squashing treatment allowed for a higher surface area in contact with the medium, resulting in a faster dissolution rate.

Table 5. pomegranate peel extract jelly (PPEJ) average weight and ellagic acid content.

Formula	Weight average (mg)	Ellagic acid content (mg)
PPEJ-A	848.50 ± 10.07[a]	3.83 ± 0.09
PPEJ-B	851.55 ± 12.16[a]	4.15 ± 0.22
PPEJ-C	852.45 ± 11.42[a]	4.16 ± 0.20

PPEJ weight average data are expressed as mean ± SD (n = 30). The ellagic content for each PPEJ formula is expressed as mean ± SD (n = 3). The data followed with common letters are not significantly different by ANOVA at a 5% level of significance ($p<0.05$).

Table 6. Textural properties of pomegranate peel extract jelly (PPEJ).

Formula	Hardness (g)	Springiness (mm)	Cohesiveness	Chewiness (N.mm)
PPEJ-A	2652.07 ± 200.85[a]	0.85 ± 0.04[a]	0.27± 0.04[a]	607.16 ± 90.69[a]
PPEJ-B	3463.89 ± 48.77[b]	0.91 ± 0.09[a]	0.27± 0.03[a]	886.42 ± 109.74[a]
PPEJ-C	3953.48 ± 361.61[c]	1.39 ± 1.05[a]	0.28± 0.09[a]	1856.68 ± 858.98[a]

Hardness, springiness, cohesiveness, and chewiness data are expressed as mean ± SD (n = 3). Springiness, cohesiveness, and chewiness data followed with common letters are not significantly different by ANOVA at a 5% significance level ($p<0.05$).

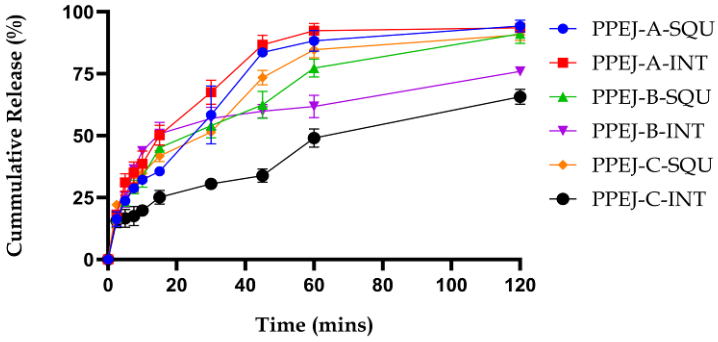

Figure 4. Cumulative release of ellagic acid at *in-vitro* dissolution study.

Data are expressed as mean ± SD (n = 3). PPEJ-SQU showed squashed PPEJ and INT showed intact PPEJ. PPEJ: pomegranate peel extract jelly; SQU: Squashed Jelly; INT: Intact Jelly.

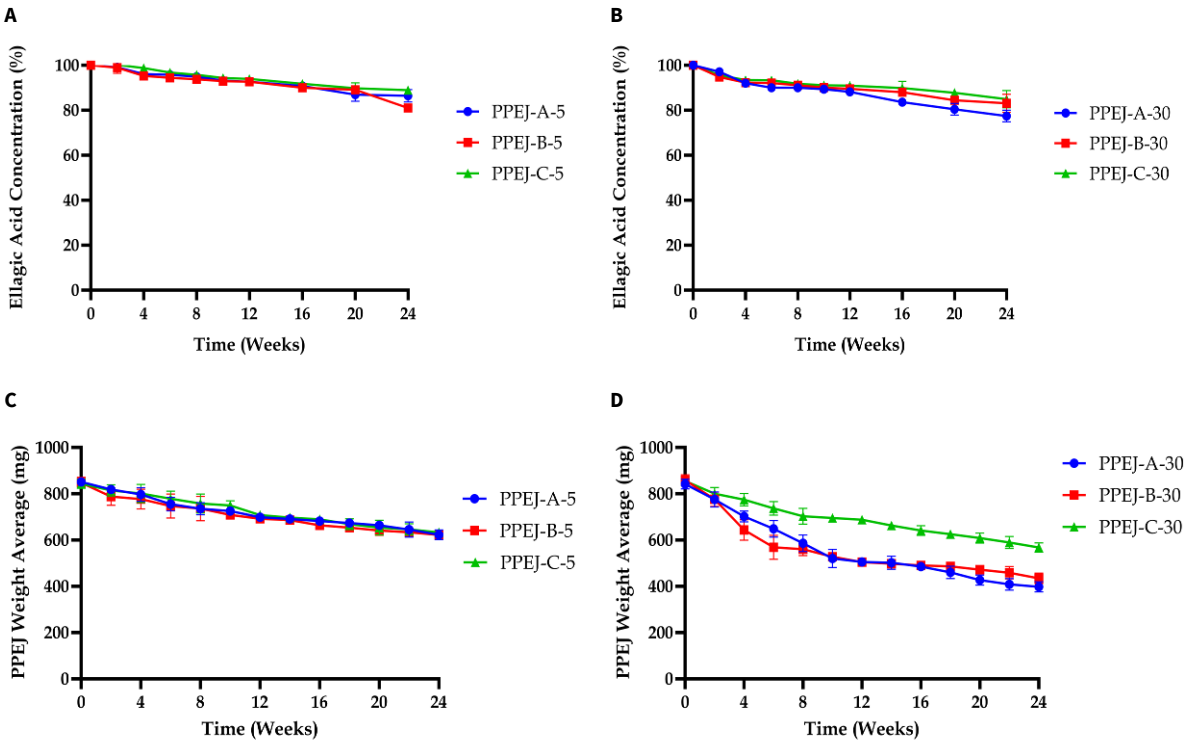

Figure 5. Stability study of pomegranate peel extract jelly (PPEJ) stored at 30°C and 5°C.

(A) Ellagic acid content during storage at 5°C for 24 weeks; **(B)** Ellagic acid content during storage at 30°C for 24 weeks; (**C**) PPEJ weight average during storage at 5°C for 24 weeks; (**D**) PPEJ weight average during storage at 30°C for 24 weeks. Ellagic acid concentration data are expressed as mean ± SD (n = 3), and PPEJ weight data are expressed as mean ± SD (n = 10).

Stability study

Fig. 5 shows PPEJ weight and ellagic acid concentration at 30°C and 5°C humidity conditions for 6 months. The loss of jelly weight during storage was reported due to syneresis, as shown by unbound excess water leaching out from the jelly matrix, resulting in shrinkage (Banerjee and Bhattacharya, 2011). This study found higher weight loss in jelly made with lower GLU-XAG-CAR concentration and stored at higher temperatures. Similar results were also stated by Elshafeey and El-Dahmy (2022), where syneresis was reported with a lower gelling agent. The syneresis rate also depended on storage temperature to promote disruption of polymer chain association and interaction between water-polysaccharides (Ako et al., 2022).

The ellagic acid concentrations in PPEJ were reduced during the storage due to leaching with water through syneresis. Furthermore, there was also a possibility that the ellagic acid reacted with the water from the syneresis.

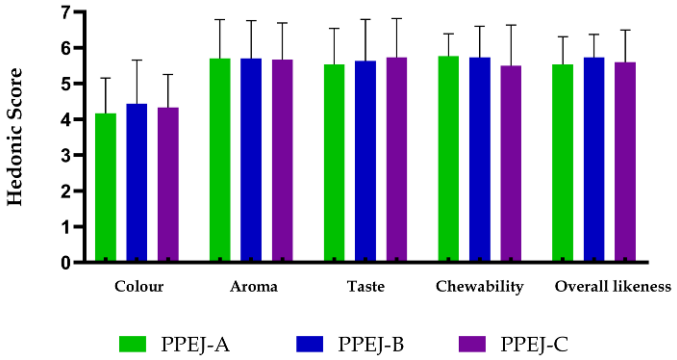

Figure 6. Hedonic attributes score of pomegranate peel extract jelly (PPEJ).

Hedonic attributes score data are expressed as mean ± SD (n = 30). The hedonic attributes scores are not significantly different by ANOVA at a 5% level of significance ($p>0.05$).

A study performed by Panichayupakaranant et al. (2010) stated that poor stability of PPE in the presence of water due to hydrolysis reaction resulted in hexahydroxydiphenic acid as the end product. In contrast, ellagic acid is also prone to oxidation due to substantial hydroxyl group content. The oxidation reaction did not affect ellagic acid due to the abundance of polyphenolic compound in the extract acting as an antioxidant.

Hedonic study

Hedonic values from color, aroma, taste, chewability, and overall likeness were analyzed, as reported in Fig. 6. PPEJ formulations were perceived by panelists as good from aroma, taste, chewability, and overall likeness aspects, while the color properties were perceived as neutral. The results showed that the improvement of GLU-XAG-CAR concentration through various PPEJ formulations was insignificant regarding the sensory attributes ($p<0.05$). In addition, the hedonic chewiness was consistent with the results of texture analysis, where panelists did not discern differences in chewability among the samples (Kayanna et al., 2022).

CONCLUSION

GLU-XAG-CAR was successfully developed to improve gel strength and swelling index properties. The successful use of GLU-XAG-CAR-7, renowned for superior gel strength value, as a gelling excipient for PPEJ, was accomplished. Meanwhile, PPEJ-C contained the best concentration and resulted in the most favorable formula, possessing the highest hardness, cohesivity, elasticity, and chewability values. Variation of GLU-XAG-CAR concentration resulted in varied texture profiles and stability parameters.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Horison R	Surini S
Concepts or ideas	x	x
Design	x	x
Definition of intellectual content	x	x
Literature search	x	x
Experimental studies	x	
Data acquisition	x	x
Data analysis	x	x
Statistical analysis	x	x
Manuscript preparation	x	x
Manuscript editing	x	x
Manuscript review	x	x

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