



Research article

Influence of natural colour blends of freeze-dried Gac aril and pulp on the quality of whey protein-mixed gelatin-based chewables

Narisara Kumkong^a, Panida Banjongsinsiri^b, Natta Laohakunjit^c, Savitri Vatanyoopaisarn^a, Benjawan Thumthanaruk^{a,*}^a Department of Agro-Industrial, Food and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand^b Expert Centre of Innovative Health Food, Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand^c School of Bioresources and Technology, King Mongkut's University of Technology, Thonburi, Bangkok, Thailand

ARTICLE INFO

Keywords:

Gac
Aril
Pulp
Chewable gummy
Whey protein
Gelatin

ABSTRACT

Gelatin gummy jelly is a chewable snack with attractive synthetic colour and flavour. The use of natural carotenoid colourant, found in Gac aril or pulp, potentially benefits consumer health. The objectives of this study were to formulate gummy prototypes designed with varying levels of gelatin, sucrose, and glucose syrup, to vary the addition of whey protein concentrate (WPC) and freeze-dried (FD) Gac aril and pulp to the selected prototype, and to investigate changes in coloured WPC-mixed gelatin gummy during storage. The prototype containing gelatin, sucrose, and glucose syrup at 10, 50, and 40%, respectively, was selected based on its hardness, gumminess, and chewiness values. The addition of WPC (0.75%) to the selected prototype increased the values of hardness, springiness, and gumminess but reduced the values of cohesiveness and chewiness. Coloured WPC-mixed gelatin gummy with blends (0.5 g/100 g) of FD Gac aril and pulp at a ratio of 75:25 appeared yellow-orange and received the highest acceptance score. The quality of coloured WPC-mixed gelatin changed to a dull colour and a softer texture gel during storage. Therefore, Gac-coloured WPC-mixed gelatin gummy improvement for colour and texture qualities should be of concern for shelf-life stability.

1. Introduction

To date, the trend of using natural colourants in food products has been increasing. The colourful pigments known as carotenoids yield attractive yellow, orange, and red colours. According to BCC Research, the global market demand for carotenoids increased by 5.7% annually and is estimated to reach \$2.0 billion by 2022 (McWilliams, 2018). Carotenoids are one of the additive ingredients in food, feed, and dietary supplements as natural colourants, flavour, and nutrition (McWilliams, 2018; Nagarajan et al., 2017). Among natural sources of carotenoid, research for carotenoid-rich plant resources has now confirmed that Gac (*Momordica cochinchinensis* Spreng) is one of the most high-potential fruits with high contents of carotenoids, especially lycopene, with content more significant than in tomatoes, watermelon, guava, or pink grapefruit (Aoki et al., 2002). From the whole Gac fruit, researchers are much interested in aril, rather than pulp, seed, or peel, with quantities of lycopene and beta carotene in the ranges of 0.380–3.728 and 0.080–0.836 mg/g (Nhung et al., 2010; Vuong et al., 2006; Ishida et al.,

2004; Aoki et al., 2002). The lycopene level is 70 times greater than in tomatoes (Oanh et al., 2017; Vuong et al., 2006). For the benefit of a natural colourant, glutinous rice is coloured with fresh Gac aril called "xoi Gac," and sold in Vietnam for special celebrations (Vuong, 2000; Kubola and Siriamornpun, 2011; Rodriguez-Amaya, 2016; Müller-Maatsch et al., 2017). This healthy foodstuff contains beta-carotene at 3.5–5.0 mg per serving (Vuong, 2000; Müller-Maatsch et al., 2017). From a medicinal perspective, the beta carotene in Gac aril can potentially cure vitamin A deficiency for vision and increase blood plasma level (Vuong et al., 2002). Given the prominent phytonutrients, commercial Gac products including Gac aril in the form of powder, puree, concentrate, frozen, juice, Gac oil in a capsule, Gac fruit jam, tea, biscuits, soap, skin balm, Gac seed alcohol, and Gac fruit enzymes, are mostly manufactured in Vietnam, China, and Thailand (Do et al., 2019). However, only a few studies on Gac pulp (Kubola and Siriamornpun, 2011; Chusak et al., 2020) and Gac peel (Kubola and Siriamornpun, 2011) as food ingredients have reported. Freeze drying is a generally practiced drying technique for retaining crucial bioactive components in Gac. Tran et al. (2008)

* Corresponding author.

E-mail address: benjawan.t@sci.kmutnb.ac.th (B. Thumthanaruk).

reported that freeze-dried Gac aril delivered the highest carotenoid level compared with other dried Gac samples.

Gelatin chewable gummy is a favoured snack product in which the attractive features derive from colour, product shape, and flavour. Concerning the ingredients used, gelatin functions as a gel network and dictates the gelling properties (Benjakul et al., 2012). Sucrose and glucose syrup give sweetness and stabilize the gel (Tau and Gunasekaran, 2016); however, combining these two ingredients yields high cohesiveness, gumminess, and chewiness values unfavourable to eating quality for consumers. As alternative sweeteners, xylitol or stevia can be substituted, and noticeable gel strength changes occur with increased xylitol or stevia content (Cai et al., 2017). Whey protein products, such as whey protein concentrate and whey protein isolate, deliver excellent functionality and nutritional properties in food-based structures, such as gel, emulsion, and foam (Damodaran, 2005; Zúñiga et al., 2011). Mixing of whey protein (5 or 10% w/w) and gelatin (5% w/w) influences gel setting characteristics (Devi et al., 2014), and the stability of beta carotene emulsion produced by whey protein mixed with beet pectin (Xu et al., 2012). The addition of synthetic colourants of red and yellow shade enhances consumer perception and satisfaction (Feketea and Tsaouri, 2017). Although artificial colours generally offer more appeal, with their brighter shades than natural colour, the consumer choices for natural colourants added to food are often acceptable. The soft and chewy gelatin with natural colourants has been limited in the market.

Therefore, this study aimed at investigating the feasibility of producing Gac-coloured WPC-mixed gelatin. The objectives were to formulate gummy prototypes designed by constrained mixture design, varying gelatin, sucrose, and glucose syrup levels to determine the selected prototype's quality changes after adding WPC and FD Gac aril and pulp, and to assess changes in coloured WPC-mixed gelatin gummy during storage. With the benefit of carotenoids in Gac, resulting in yellow-orange to orange-red colour, the vivid colouring in gelatin gummy could enhance Gac's use and produce functional gelatin gummy with a soft and chewy texture.

2. Material and method

2.1. Materials

Frozen aril and pulp from Gac fruits were obtained from Hopia Co., Ltd. Maltodextrin (MD) 10 DE, and gelatin bloom 250 was purchased from Phacobic Co., Ltd (Thailand). Whey protein concentrate (WPC) was purchased from Hilmar Ingredient Co., Ltd. "Mitrphol®" brand sucrose was purchased from a supermarket in Bangkok, and mixed-berry flavouring was purchased from Getece, Co., Ltd, Thailand. All other chemicals and solvents in this study were of analytical grade.

2.2. Survey and analysis of commercial chewable gelatin gummy

Eight samples of chewable gelatin gummy sold in supermarkets located in Bangkok, Thailand, were selected for sensory tasting and texture analysis.

2.3. Formulation of chewable gelatin gummy prototype designed by constrained mixture design

The constrained mixture design applied for producing nine gummy prototypes were composed of gelatin (10–15%), glucose syrup (40–65%), and sucrose (40–65%), with a total of 72.5 g/100 g (by weight). The concentrations of citric acid and water were set as constants at 1.75 and 25.75 g/100g. Sodium benzoate and mixed-berry flavouring used were at concentrations of 0.1 g/100 g and 0.13 g/100 g in all formulas. Chewable gelatin gummy was prepared by mixing a gelatin powder with water and heating it in a microwave oven for 1.5 min. Sucrose and glucose syrup was then solubilized in water and heated at 65–70 °C. Next, the gelatin solution and sugar solution were mixed for 3 min, adding citric acid,

sodium benzoate, mixed-berry flavouring, and stirred until the solution became clear. The resulting solution was then poured into silicone moulds and kept at room temperature until the gel was set. The resulting gelatin gummy was removed from the moulds and kept in a polypropylene bag for analysis.

2.4. Preparation of freeze-dried (FD) Gac aril and Gac pulp

Preparation of FD Gac aril and Gac pulp samples was performed according to the method described by Kumkong et al. (2018). Briefly, after thawing frozen Gac aril and Gac pulp to room temperature, separating out seeds, and filtrating, each aril or pulp sample was mixed with maltodextrin DE 10 at 5 g/100 g (w/w) and subjected to freeze drying (Thailand Institute of Scientific and Technology Research, (TISTR), Thailand). The operational conditions were condenser temperature of -20 °C and pressure of 250 Pa for 48 h. Powder samples of FD aril and FD pulp were packed separately in aluminum foil and kept at 4 °C before use.

2.5. Variation of WPC and FD Gac to gelatin gummy

WPC was added to selected prototype formula one at 0.5, 0.75, and 1.0 g/100 g of total gelatin. The optimum and the better results achieved by adding 0.75% WPC were selected for further study. The FD aril used was varied at 0.5, 1.0, and 1.5 g/100 g of WPC-gelatin gummy, and blends of FD aril and FD pulp were varied at 100:0, 75:25, 50:50, and 25:75 w/w with a total of 0.5 g per 100 g sample. The other ingredients used were applied in the same amounts, as shown in section 2.3. The shelf life study of the coloured WPC-mixed gelatin samples was carried out at 25 °C for 8 weeks.

2.6. Analysis

2.6.1. Physicochemical properties

All formulated test gelatin gummy samples were analyzed. The moisture content was determined, according to (Horwitz and Latimer, 2005). A water activity meter (Decagon Devices, USA) was used to measure water activity (a_w). The pH values were determined using a pH meter. An auto-titrator (Mettler Toledo, USA) was used to titrate each sample with 0.1 N NaOH. The value of % TA was reported as citric acid. Measurement of total soluble solids was performed using a hand refractometer (Atago, Japan). The analysis of FD Gac aril and Gac pulp powders were β -carotene and lycopene content according to the method described by Ishida et al. (2004). DPPH assay was performed following the method described by Jang and Kim (2014). Scavenging activity of DPPH radical was calculated according to the following formula (1):

$$\% \text{ Radical scavenging} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (1)$$

The colour of FD gac powder and test gelatin samples was measured using a colourimeter (Colour Quest XE, UK.). Hunter colour values of L^* , a^* , and b^* were expressed in chroma, hue angle (H°), and the total colour difference (ΔE), according to formulas (2), (3), and (4) below:

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$H^\circ = \tan^{-1} (b^*/a^*) \quad (3)$$

$$\text{Total colour difference } (\Delta E) = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (4)$$

The texture properties of gummy samples were measured using a texture analyzer (Stable Micro Systems, Ltd., MA, USA.). The gummy samples were cut to a size of 1 cm (W) x 3 cm (L). The setting parameters were: pre-test speed (1 mm/s), test speed (5 mm/s), post-test speed (5 mm/s), distance format (10 mm), and trigger force (0.05 N). Each treatment was measured in five replicates and reported in mean and standard deviation. Texture profile analysis (TPA) yields maximum forces to deform the gel during the first compression, indicating hardness

1 (N) and hardness 2 (N), which are related to the first bite and second bite (Van den Berg et al., 2008). Cohesiveness refers to the visco-elasticity of a sample. Springiness is the height ratio that the sample springs back to after the first compression to the maximum deformation. Gumminess (N) is the force required to break down a semi-solid food for swallowing. Chewiness (N.mm) is the energy required to chew solid food into a state appropriate to swallowing (Thongudomporn et al., 2015).

2.6.2. Sensory evaluation

Gummy gelatin samples of commercial products, prototypes, WPC-mixed gelatin, and coloured WPC-mixed gelatin were sensory tasted by 30 panellists familiar with gummy gelatin products and aged 20–30 years. Informed consent was obtained from all panellists in this experiment, and explanations were given to all panellists. A 7-point hedonic scale was used in this sensory test on colour, texture, flavour, sweetness, sourness, stiffness, and overall acceptance.

2.7. Statistical analysis

For the formulation of chewable gelatin gummy jelly, a constrained mixture design was applied. The analysis of variance (ANOVA) and significant differences of mean value using Duncan's multiple range test was analyzed using Minitab 19.0 software.

3. Results and discussion

3.1. Survey of commercial chewable gelatin gummy

The eight surveyed commercial products exhibited different textural profiles. The studied products were seven locally-made products and one imported product. Looking closely at the ingredient list on the labels, the quantities of gelatin, sucrose, and glucose syrup were in the ranges of 5–12%, 25–64.5%, and 24–51%, respectively (Figure 1). Citric acid contained in all samples for acidity, and sodium benzoate was used as a preservative. Apart from this information, other ingredients were not identified, as they may be considered as trade secrets. Two products (samples A and E) had high gelatin content, greater than 10% (Figure 1). Sample E, containing 15% of gelatin, displayed the highest hardness, gumminess, and chewiness values. Although the high gelatin content results in increased hardness, the increased amount of glucose syrup also affects hardness, gumminess, and chewiness. With a gelatin level of 8%,

sample F showed a more critical textural change with glucose syrup content than sucrose content (sample H). In such gummy products, gelatin is responsible for cold temperature and thermoreversible gel characteristics (Lin et al., 2017). The gel structure occurs due to hydrogen bonds and hydrophobic and ionic interactions (Benjakul et al., 2012), and is affected by the percentage of gelatin and sugar concentration, establishing the textural or sensory properties of the gelatin gel. Among the different commercial gelatin gummy brands with varying ingredients and texture results, sample H, with gelatin (8%), sucrose (62.5%), and glucose syrup (25%), was selected and received 7-point hedonic scores on appearance, colour, flavour, sweetness, sourness, stickiness, and overall acceptability of 6.13 ± 0.62 , 6.13 ± 0.72 , 5.87 ± 0.62 , 5.93 ± 0.57 , 5.67 ± 0.70 , 5.47 ± 1.02 , and 5.93 ± 0.57 .

3.2. Effect of gelatin, sucrose, and glucose syrup on the quality of chewable gelatin gummy prototypes

The nine formulas of developed gelatin gummy showed increased gelatin content from 10 to 15% of 72.5 g/100g, significantly increased hardness, gumminess, and chewiness (Figure 2). Concerning texture profile results, chewiness is highest as the gelatin increased to 15%, exhibiting a much higher hardness and energy to chew before swallowing. Apart from gelatin, the impact on gel texture is also associated with glucose syrup. Glucose syrup prevents sucrose crystallization; however, the high content of glucose syrup increases stickiness. All nine formulas also presented slight differences in TSS (70.99–78.31 °Brix), pH (3.06–3.47), %TA (4.57–6.50), and aw (0.77–0.83). Although the prototypes containing 15% gelatin (formula 7-9) exhibited higher textural values of hardness, gumminess, and chewiness, the sensory results did not show high acceptance scores. Of all nine formulas, the hedonic scores of appearance, colour, flavour, sweetness, sourness, stickiness, and overall acceptance were 5.17 ± 1.29 – 5.80 ± 1.08 , 5.07 ± 1.15 – 5.60 ± 0.92 , 4.17 ± 1.42 – 4.67 ± 1.58 , 4.70 ± 1.04 – 5.10 ± 1.16 , 4.63 ± 0.98 – 5.23 ± 1.20 , 4.67 ± 1.42 – 5.40 ± 1.38 , and 4.65 ± 1.61 – 5.33 ± 1.14 . Hence, the prototype selected for further study was formula 1, with gelatin, sucrose, and glucose syrup of 10, 50, and 40 %, and the highest sensory scores of appearance (5.80 ± 1.08), colour (5.60 ± 0.92), flavour (4.67 ± 1.58), sweetness (5.10 ± 1.16), stickiness (5.40 ± 1.38), and overall acceptance (5.33 ± 1.14). Sourness, however, was not maximum, at 5.07 ± 1.29 . The overall acceptance scores indicated "like slightly" to

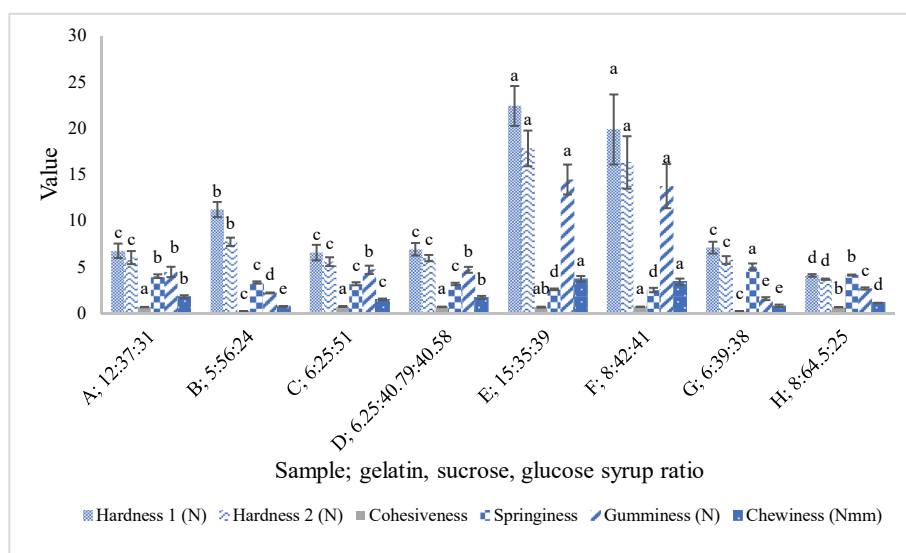


Figure 1. Textural properties of eight surveyed products with different ratios of gelatin, sucrose, and glucose syrup. Note: Differences in small letters of the same parameter show significant differences (p < 0.05).

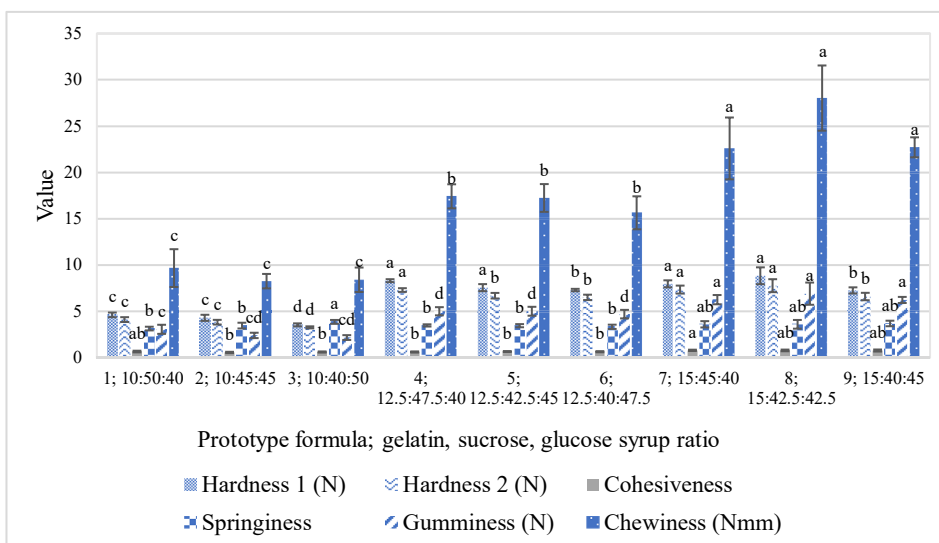


Figure 2. Textural properties of nine prototypes with different ratios of gelatin, sucrose, and glucose syrup. Note: Differences in small letters of the same parameter show significant differences ($p < 0.05$).

"like moderately." The texture profile results of prototype formula 1 were within the range required for commercial products.

3.3. Effect of addition of WPC and FD Gac powders on gummy gelatin quality

3.3.1. Texture

The addition of WPC at all levels (0.5, 0.75, and 1.0 g per 100 g gelatin) to gelatin gels changes the gel texture profile. With increased WPC content, the WPC-gelatin mixed gel exhibited only increased springiness (3.60 ± 0.16 – 3.89 ± 0.21), but decreased hardness 1 (14.07 ± 1.32 – 11.02 ± 0.21 N), hardness 2 (11.94 ± 1.16 – 9.30 ± 0.32 N), cohesiveness (0.61 ± 0.10 – 0.54 ± 0.02), gumminess (8.49 ± 0.84 – 5.91 ± 0.33 N), and chewiness (3.11 ± 0.27 – 2.29 ± 0.07 Nmm) (Figure 3). Although two gelatin proteins and WPC can individually affect gel property, the mix of these two proteins only supports gummy springiness. Although the WPC addition to gelatin-based gummy increased the solid

content, the results also agreed with Devi et al. (2014) in which a low concentration of whey protein mixed with gelatin developed a low-density network during heating, resulting in the formation of a triple helix structure of gelatin in its interstices. The interaction of the intra- and inter-molecular covalent crosslinks bonding from lysine and hydroxylysine (Hyl) residues and aldehyde derivatives within gelatin (Bateman et al., 1996) might be disrupted by alpha-lactalbumin and beta-lactoglobulin in WPC (Heino et al., 2007). After comparing samples at the three WPC levels, the application of 0.75 g/100 g was selected for further study.

For the colour feature, the addition of FD Gac aril at all levels to WPC-gelatin gel benefits colour shade but significantly changes almost all texture properties. Increased FD Gac aril to WPC-mixed gelatin reduced hardness 1 (8.28 ± 0.61 – 7.02 ± 0.21 N), hardness 2 (7.26 ± 0.46 – 5.92 ± 0.33 N), cohesiveness (0.57 ± 0.06 – 0.52 ± 0.09), gumminess (4.72 ± 0.71 – 3.64 ± 0.60 N), and chewiness (1.85 ± 0.32 – 1.56 ± 0.35). However, the value of springiness was slightly increased without significant

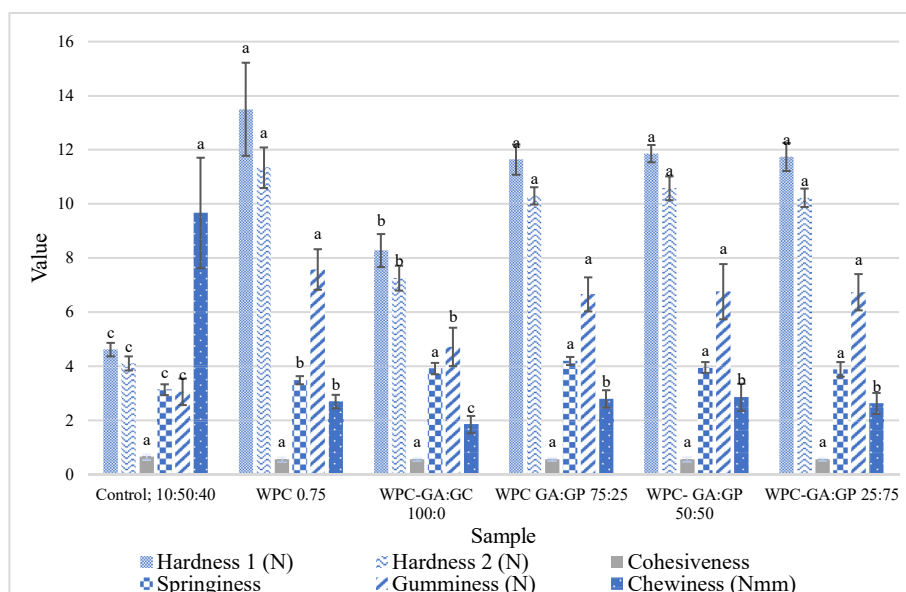


Figure 3. Textural properties of WPC and Gac (Gac aril; GA, Gac pulp; GP) added to gelatin samples. Note: Differences in small letters of the same parameter show significant differences ($p < 0.05$).

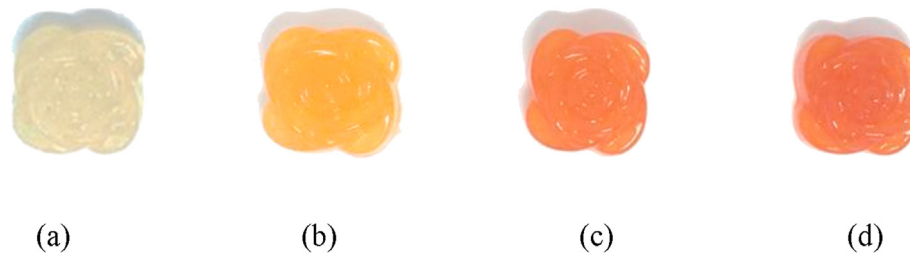


Figure 4. WPC-mixed gelatin gummy colored with FD Gac aril 0 g/100 g (control) (a); 0.5g/100g (b); 1g/100g(c); 1.5g/100g (d).

differences (3.92 ± 0.21 – 4.24 ± 0.30) (Figure 3). The noticeable reduction in texture properties was found mostly in the sample with the maximum FD Gac aril (1.5g/100g). Colouring by Gac in WPC-mixed gelatin gummy might cause the interaction of betacarotene bound to β -lactoglobulin in whey protein (Allahdad et al., 2018). The hydrophobic compounds in the Gac aril, including lycopene, beta carotene, and fat, might interfere with the hydrophilic compound in a gel matrix. The Gac samples added to food products also affect texture properties, depending on the type of Gac and food product. The findings of Chusak et al. (2020) revealed that the significantly increased hardness of pasta with unripe and ripe Gac fruit powder is due to the hydrophobic effect of dietary fibre on the pasta starch.

3.3.2. Colour

Colour is a necessary physical property for gummy snacks that enhances consumer buying. The WPC-mixed gelatin prototype displayed a light pale colour (Figure 4; a). In our previous work, we produced FD Gac aril and FD Gac pulp with 5% maltodextrin. Table 1 shows the quality of the FD gac samples used. The pronounced bioactivity of carotenoids of FD Gac aril, mainly beta carotene, lycopene, and antioxidant, agreed with Mai et al. (2013) and Kumkong et al. (2018). Concerning the benefit of the orange-red colour of aril, increased FD Gac aril at 0.5, 1.0, and 1.5 g/100 g to WPC-mixed gelatin gummies resulted in increased redness (a^* ; 2.40 ± 0.34 – 3.26 ± 0.03), yellowness (b^* ; 1.84 ± 0.27 – 1.52 ± 0.17), and chroma (3.03 ± 0.42 – 3.60 ± 0.010), but not lightness (L^* ; 41.13 ± 0.05 – 38.25 ± 0.52) or hue angle (h° ; 37.51 ± 2.48 – 24.89 ± 2.30). The products exhibit a light yellow-orange shade of colour (Figure 4: b-d). Given the light yellow in FD Gac pulp, the experiments further investigated the colour obtained after adding blends of FD aril and FD pulp with a total of 0.5 g/100 g at ratios of 100:0, 75:25, 50:50, and 25:75. Results show that the increased proportions of Gac pulp used result in a decrease in lightness (L^* ; 41.13 ± 0.05 – 38.71 ± 0.40), redness (a^* ; 2.40 ± 0.34 – 0.06 ± 0.04), yellowness (b^* ; 1.84 ± 0.27 – 0.23 ± 0.08), and

chroma (3.03 ± 0.42 – 0.24 ± 0.06), but an increase in hue angle (h° ; 37.51 ± 2.48 – 73.37 ± 16.58). However, these products appear in a yellow shade (Figure 5; a-d) rather than an orange-red shade with increased Gac aril (Figure 4; a-d).

3.4. Changes in quality of coloured WPC-mixed gelatin gummy during storage

3.4.1. Texture

For the study of shelf-life, the addition of FD Gac aril and pulp at 75:25 with a total of 0.5% colourant added to WPC-gelatin mixed gummy stored at room temperature changed the gel texture of WPC-mixed gelatin gummies. The values of texture parameters were reduced in hardness 1, hardness 2, gumminess, and chewiness, but not in cohesiveness or springiness values (Figure 6). Also, for appearance, the gelatin gels softened gradually with extended storage time. The gradual release of the liquid phase from the gel structure results in softening of the WPC-mixed gelatin gummy texture resulting in the large degradation of gel and fracture properties. This phenomenon agrees with the instability of gelatin gel results of Lin et al. (2017) and Van den Berg et al. (2008). Given the study results, the stability of the texture attribute of Gac-coloured WPC-mixed gelatin gummy should be of more concern than the colour perspective during storage.

3.4.2. Colour

Coloured WPC-mixed gelatin gummy develops a dull appearance during storage. Significant differences in lightness (L^*) after two weeks of storage occurred, with decreases from 85.01 ± 0.05 to 43.75 ± 0.10 and gradually reduced to 40.64 ± 0.02 at 7 weeks of storage. The values of redness (a^*) and yellowness (b^*) increased from -0.75 ± 0.01 – 0.15 ± 0.02 and 0.18 ± 0.0 – 1.48 ± 0.34 . After calculation, the values of chroma, hue angle, and total colour differences changed from 0.77 ± 0.01 – 1.48 ± 0.34 , 13.50 ± 0.83 – 84.14 ± 1.43 , and 0 – 44.40 ± 0.06 at the end of

Table 1. Physiochemical characteristics of freeze-dried Gac sample with 5% maltodextrin used.

Characteristic	FD Gac aril	FD Gac pulp
Moisture (%)	3.32 ± 0.20^A	3.62 ± 0.26^A
Beta carotene (mg/g)	36.51 ± 0.12^A	1.34 ± 0.01^B
Lycopene (mg/g)	23.12 ± 0.53^A	4.58 ± 0.10^B
DPPH radical scavenging Activity (%)	79.26 ± 0.49^A	69.76 ± 0.19^B
Colour		
L^*	62.75 ± 0.64^B	67.61 ± 0.08^A
a^*	19.80 ± 0.51^A	14.61 ± 0.05^B
b^*	20.86 ± 0.75^B	33.12 ± 0.08^A
Chroma	65.81 ± 0.46^B	78.15 ± 0.23^A
Hue angle	46.48 ± 0.67^B	70.31 ± 0.25^A
Total colour difference ^{ns}	46.48 ± 0.97	47.96 ± 0.35

Values are expressed as mean \pm standard deviation ($n = 3$) and calculated on a dry weight basis.

Numbers with different capital letter superscripts in each row are significant differences ($p < 0.05$).

The total colour difference values were calculated L_0 from FD Gac aril and FD Gac pulp with 0% maltodextrin.

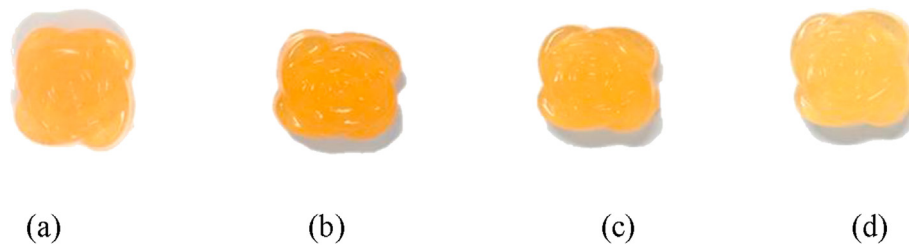


Figure 5. Color of WPC-mixed gelatin gummy with 0.5 g/100g of Gac powder added at three ratios of FD aril: FD pulp: 100:0 (a); 75:25 (b); 50:50 (c); 25:75 (d).

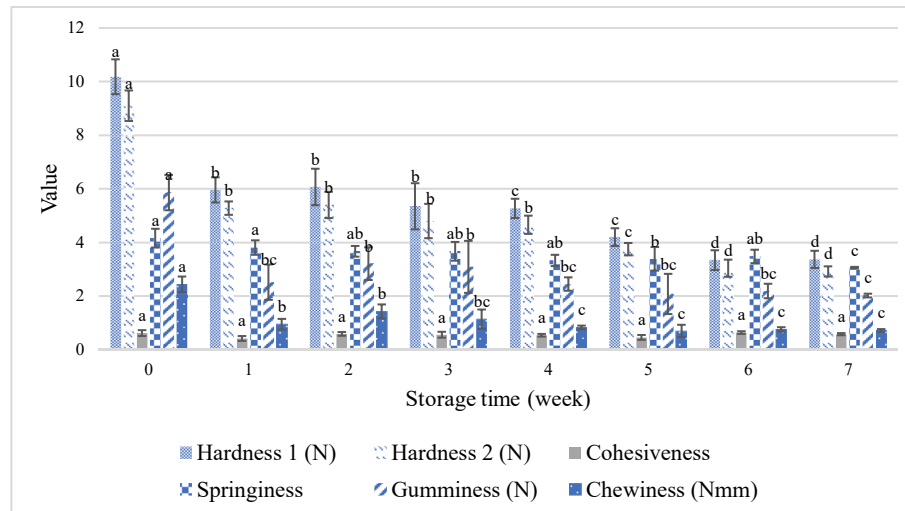


Figure 6. Textural properties of Gac colored WPC mixed gelatin during storage. Note: Differences in small letters of the same parameter show significant differences ($p < 0.05$).

storage time. Factors causing the colour changes may be the Maillard reaction, where the free amino acid reacts with reducing sugar and yields a brown shade of melanoid compounds (Van Boekel, 1998). The condition of high water activity at approximately 0.80 of WPC-mixed gelatin gummy may facilitate the Maillard reaction. Concerning the resulting brown colour, the bioactive compounds of beta carotene and lycopene, despite both showing antioxidant activity and beta carotene showing pro-vitamin A activity, may undergo degradation and isomerization of lycopene as affected by heat, light, and storage (Lee and Chen, 2002; Nguyen and Schwartz, 1999). The gummy samples did not last beyond eight weeks due to the excessive amounts of yeast and mould growth, despite the use of sodium benzoate as a preservative.

The WPC-mixed gelatin gummy coloured with FD Gac powders received higher hedonic scores than the control with no colourant added. The scores of WPC-mixed gelatin gummy coloured with all FD Gac arils added indicated no significant differences in terms of colour (5.90 ± 0.70 - 5.97 ± 0.95), texture (5.95 ± 0.93 - 5.98 ± 0.05), flavour (5.53 ± 0.72 - 5.80 ± 0.95), sweetness (5.50 ± 0.81 - 5.90 ± 1.04), sourness (5.47 ± 0.80 - 5.67 ± 1.11), stickiness (5.60 ± 0.95 - 5.87 ± 1.06), or overall acceptance (5.47 ± 0.85 - 6.07 ± 0.85). The addition of 0.5 g/100 g FD Gac aril received higher likeness than the other formula. When the blend of FD Gac powders at different ratios of aril and pulp was added to the WPC-mixed gelatin gummy, results showed that the use of 0.5 g/100 g Gac powder with mixed FD aril and FD pulp at the ratio of 75:25 received the highest overall likeness score of 6.13 ± 0.67 on the 7-point hedonic scale. The values of all formulas showed no significant differences. Thus, the bioactive gelatin gummy filled with FD Gac can be favourably used for gelatin gummy to benefit from Gac's antioxidant activity. The developed chewable gelatin gummy is also needed for patients with dysphagia (Dille et al., 2018). However, the cost of producing natural Gac colourant and fading of colour must be taken into consideration.

4. Conclusion

The addition of WPC (0.75%) to the gummy gelatin prototype containing 10% gelatin, 50% sucrose, and 40% glucose syrup increased the solid composition of gummy, resulting in increased hardness 1, hardness 2, springiness, and gumminess. The WPC-mixed gelatin gummy coloured with FD Gac aril and pulp's blends. The coloured WPC-mixed gelatin gummy at 0.5% of Gac aril and Gac pulp (ratio 75:25) gradually changed to a dull colour and reduced hardness during storage. Future research is needed for quality improvement based on gel and colour stability of Gac coloured mixed chewy gels of WPC and gelatin.

Declarations

Author contribution statement

Narisara Kumkong: Performed the experiments; Analyzed and interpreted the data.

Panida Banjongsinsiri, Natta Laohakunjit: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Savitri Vatanyoopaisarn: Conceived and designed the experiments.

Benjawan Thumthanaruk: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by King Mongkut's University of Technology North Bangkok (KMUTNB-60-GOV-2560A11902083) and Thailand Institute of Scientific and Technological Research (TISTR) (6213105010).

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Allahdad, Z., Varidi, M., Zadmand, R., Saboury, A.A., Haertlé, T., 2018. Binding of β -carotene to whey proteins: multi-spectroscopic techniques and docking studies. *Food Chem.* 277, 96–106.
- Aoki, H., Kieu, N.T., Kuze, N., Tomisaka, K., Chuyen, V.N., 2002. Carotenoid pigments in GAC fruit (*Momordica cochinchinensis* Spreng). *Biosci. Biotechnol. Biochem.* 66 (11), 2479–2482.
- Bateman, J.R., Lamandé, S.R., Ramshaw, J.A.M., 1996. In: Comper, W.D. (Ed.), *Extracellular Matrix, Molecular Components and Interactions*. Harwood Academic Publishers, UK, pp. 22–27.
- Benjakul, S., Kittiphattanabawon, P., Regenstein, J.M., 2012. Fish gelatin. In: Simpson, B.K., Nollet, L.M.L., Toldrae, F. (Eds.), *Food Biochemistry and Food*. John Wiley & Sons Inc., Ames, IA, pp. 388–405.
- Cai, L., Feng, J., Regenstein, J., Lv, Y., Li, J., 2017. Confectionery gels: effects of low calorie sweeteners on the rheological properties and microstructure of fish gelatin. *Food Hydrocoll.* 67, 157–165.
- Chusak, C., Chanchunyawat, P., Chumnumduang, P., Chantarasinlapin, P., Suantawee, T., Adisakwattana, S., 2020. Effect of gac fruit (*Momordica cochinchinensis*) powder on *in vitro* starch digestibility, nutritional quality, texture and sensory characteristic of pasta. *LWT-Food Sci Technol.* 118, 108856.
- Damodaran, S., 2005. Protein stabilization of emulsions and foams. *J. Food Sci.* 69, R54–R66.
- Devi, A.F., Buckow, R., Hemar, Y., Kasapis, S., 2014. Modification of the structural and rheological properties of whey protein/gelatin mixtures through high pressure processing. *Food Chem.* 156, 243–249.
- Dille, M.J., Magnus, N.H., Dragnet, K.L., 2018. Bioactively filled gelatin gels: challenges and opportunities. *Food Hydrocoll.* 76, 17–29.
- Do, T.V.T., Fan, L., Suhartini, W., Girmatsion, M., 2019. Gac (*Momordica cochinchinensis* Spreng) fruit: a functional food and medicinal resource. *J. Funct. Foods.* 62, 103512.
- Feketea, G., Tsaouri, S., 2017. Common food colourants and allergic reactions in children; Myth or reality? A-Review. *Food Chem.* 230, 578–588.
- Heino, A., Uusi-Rauva, J.O., Rantamäkim, P.R., Tossavainen, O., 2007. Functional properties of native and cheese whey protein concentrate powders. *Inter. J. Dairy Technol.* 60 (4), 277–285.
- Horwitz, W., Latimer, G.W., 2005. *Official Methods of Analysis of AOAC International*. Gaithersburg, MD.
- Ishida, B.K., Turner, C., Chapman, M.H., McKeon, T.A., 2004. Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. *J. Agric. Food Chem.* 52 (2), 274–279.
- Jang, M., Kim, G.-H., 2014. Antioxidant activity and HPLC analysis of lycopene, β -carotene and α -tocopherol from Geuk (*Momordica cochinchinensis* Spreng) fruit. *J. Int. Sci. Publ.: Agric. Food.* 2, 430–438.
- Kubola, J., Siriamornpun, S., 2011. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai Gac (*Momordica cochinchinensis* Spreng). *Food Chem.* 127, 1138–1145.
- Kumkong, N., Thumthanaruk, B., Banjongsinisiri, P., 2018. Factors affecting stability of lycopene and β -carotene from Gac aril powder by freeze drying. *J. Food Sci. Agric. Technol.* 4, 73–78.
- Lee, M.T., Chen, B.H., 2002. Stability of lycopene during heating and illumination in a model system. *Food Chem.* 78, 425–432.
- Lin, L., Regenstein, J.M., Lv, S., Lu, J., Jiang, S., 2017. An overview of gelatin derived from aquatic animals: properties and modification. *Trends Food Sci. Technol.* 68, 102–112.
- Mai, H.C., Truong, V., Haut, B., Debaste, F., 2013. Impact of limited drying on *Momordica cochinchinensis* Spreng. aril carotenoids content and antioxidant activity. *J. Food Eng.* 118, 358–364.
- McWilliams, A., 2018. *The Global Market for Carotenoids*. BCC Research Report Overview. BCC Research LLC. <https://www.bccresearch.com/market-research/food-and-beverage/the-global-market-for-carotenoids.html>.
- Müller-Maatsch, J., Sprenger, J., Hempel, J., Kreiser, F., Carle, R., Schweiggert, R.M., 2017. Carotenoids from gac fruit aril (*Momordica cochinchinensis* [Lour.] Spreng.) are more bioaccessible than those from carrot root and tomato fruit. *Food Res. Int.* 99, 928–935.
- Nagarajan, J., Ramanan, R.N., Raghunandan, M.E., Galanakis, C.M., Krishnamurthy, N.P., 2017. *Carotenoids. Nutraceutical and Functional Food Components. Effects of Innovative Processing Techniques*. Academic Press, San Diego, CA, pp. 259–296.
- Nguyen, M.L., Schwartz, S.J., 1999. Lycopene: chemical and biological properties. *Food Technol.* 45–53.
- Nhung, D.T.T., Bung, P.N., Ha, N.T., Phong, T.K., 2010. Changes in lycopene and beta carotene contents in aril and oil of gac fruit during storage. *Food Chem.* 121, 326–331.
- Oanh, H.T., Nhung, H.T., Tuyen, N.D., Van, L.T.K., Trung, T.H., Ha, H.M., 2017. Extraction of lycopene from gac fruit (*Momordica cochinchinensis* Spreng) and preparation of nanolycopene. *Vietnam J. Chem.* 55 (6), 761–766.
- Rodriguez-Amaya, D.B., 2016. Natural food pigments and colourants. *Curr. Opin. Food Sci.* 7, 20–26.
- Tau, T., Gunasekaran, S., 2016. Thermorheological evaluation of gelation of gelatin with sugar substitutes. *LWT-Food Sci. Technol.* 69, 570–578.
- Thongdomporn, U., Chongsuvivatwong, V., Geater, A.F., 2015. Classification of common foods consumed by Thais based on textural properties. *Songklanakarin Dent. J.* 3 (2), 45–52.
- Tran, T.H., Nguyen, M.H., Zabar, D., Vu, L.T.T., 2008. Process development of Gac powder by using different enzymes and drying techniques. *J. Food Eng.* 85 (3), 359–365.
- Van Boekel, M.A.J.S., 1998. Effect of heating on Maillard reactions in milk. *Food Chem.* 62 (4), 403–414.
- Van den Berg, L., Carolas, A.L., Van Vliet, T., Van der Linden, E., Van Boekel, M.A.J.S., Van de Velde, F., 2008. Energy storage controls crumbly perception in whey proteins/polysaccharide mixed gels. *Food Hydrocoll.* 22 (7), 1404–1417.
- Vuong, T.L., 2000. Underutilized β -carotene-rich crops of Vietnam. *Food Nutr. Bull.* 21, 173–181.
- Vuong, L.T., Ducker, S.R., Murphy, S.P., 2002. Plasma β -carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (Gac). *Am. J. Clin. Nutr.* 75 (5), 872–879.
- Vuong, L.T., Franke, A.A., Custer, L.J., Murphy, S.P., 2006. *Momordica cochinchinensis* Spreng. (Gac) fruit carotenoids reevaluated. *J. Food Compos. Anal.* 19 (6-7), 664–668.
- Xu, D., Wang, X., Jiang, J., Yuan, F., Gao, Y., 2012. Impact of whey protein – beet pectin conjugation on the physicochemical stability of β -carotene emulsions. *Food Hydrocoll.* 28 (2), 258–266.
- Zúñiga, R.N., Kulozik, U., Aguilera, J.M., 2011. Ultrasonic generation of aerated gelatin gels stabilized by whey protein β -lactoglobulin. *Food Hydrocoll.* 25, 958–967.