

DEVELOPMENT OF GOAT MILK YOGURT GUMMY JELLY FORTIFIED WITH CALCIUM AND COLLAGEN

Sumalee Musika^{*}, Puridet Aupari, Witsanuwat Bunmathon, Chanida Kupradit, Araya Ranok, Seksan Mangkalan and Chompoonuch Khongla

Received: July 17, 2021; Revised: December 21, 2021; Accepted: January 14, 2022

Abstract

The aim of this work was to develop goat's milk yogurt (GMY) gummy jelly fortified with 1% calcium and various levels of collagens (1, 1.5, and 2.0%). The GMY-gummy jelly qualities were determined. The results found that GMY-gummy jelly fortified with 1% calcium and 2% collagen (T3) contained higher protein content, antioxidant activities, and all sensory attributes score than control (GMY-gummy jelly without the addition of calcium and collagen). The product qualities of T3 including pH, titratable acidity (TA), total soluble solid, Brix/Acid ratio, protein, ash, moisture, a_w , ABTS radical scavenging activity, ferric reducing antioxidant power, and metal chelating activity were 4.43, 0.76%, 57.87 g/100 g, 76.06, 10.19%, 1.60%, 29.48%, 0.8083, 13.86 mg Trolox equivalents/100 g, 20.87 mg Trolox equivalents/100 g, and 86.64 mg EDTA equivalents/100 g, respectively. In addition, GMY-gummy jelly with 2% collagen whose score was 7.62-7.87 (Like Moderately to Like Very Much), was accepted by 300 people. Moreover, its microbiological qualities did not exceed the limitation of Thai community product standard (520/2547). Our findings indicated that the developed goat's milk yogurt gummy jelly with 1% calcium and 2% collagen is potentially a good source of protein, minerals, and antioxidant substances.

Keywords: Goat's milk yogurt, Gummy jelly, Collagen, Antioxidant activity, Product qualities

Introduction

Gummy jelly is an intermediate moisture food, a confectionery product in a group of candy gel which is derived from concentrated sugar solutions, gelling agents (gelatin, starch or pectin) and other ingredients such as, acids, colorings, and flavoring (Burey *et al.*, 2009). Gummy jellies are considered to have little nutritional value and negative effects on health due to their high sugar content. Therefore, current studies have focused on developing healthy gummy jellies with reduced sugar

(Takeungwongtrakul *et al.*, 2020), or with bioactive ingredients such as, probiotics and prebiotics (Lele *et al.*, 2018), or other substances with antioxidants such as, acai berry extract, honey, propolis, and guava (*Psidium guajava*) leaf extract (Charoen *et al.*, 2015; Čižauskaitė *et al.*, 2019; Rivero *et al.*, 2020), and/or other active ingredients such as, calcium carbonate and vitamin D3 (Čižauskaitė *et al.*, 2019).

Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000, Thailand. E-mail: musika_noi@hotmail.com

Yogurt is a fermented milk product processed by using specific lactic acid bacteria (LAB) cultures namely, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Fazilah *et al.*, 2018). It is considered a healthy food because it contains viable bacteria that are considered probiotics (Hashim *et al.*, 2009). In addition, yogurt is a rich source of protein and peptides. Yogurt is traditionally made from cow's milk, however, milk from other sources has also been used to make yogurt. (De Santis *et al.*, 2019). Goat's milk is an alternative milk source suitable for the production of yogurt due to the milk's nutritional value which is higher than cow's milk, in its composition, as well as its numerous health benefits (Verruk *et al.*, 2019). Therefore, the addition of goat's milk yogurt in gummy jelly would increase the nutritional value of products. In addition, goat's milk yogurt (GMY) gummy jelly is an alternative product for patients with cow's milk allergy.

Collagen is widely employed in multifarious human applications, such as food (protein dietary supplements, carriers, and food additives), cosmetics, pharmaceutical/biomedical, and nutraceutical products. Collagen is a connective tissue protein found in skin, teeth, and bones (Nurilmala *et al.*, 2020). Collagen helps to maintain the structure of various tissues and organs. Collagen can be converted to gelatin by high temperature treatment. It has been reported that collagen and gelatin protein and/or peptides possess antioxidant activity (Nurilmala *et al.*, 2020; Wang *et al.*, 2020). Therefore, the development of GMY-gummy jelly fortified with collagen peptides will not only increase the nutritional value but also antioxidant activity of products.

The other selected active ingredient to incorporate into the goat's milk yogurt gummy jelly is calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) because it is essential for the development, growth, and maintenance of bones, where it supports their structure and hardness. Calcium is the predominant mineral incorporated into bones and teeth of the human body in the form of calcium hydroxyapatite crystals (O'Keefe *et al.*, 2016). The recommended daily intake (RDI) of calcium is 1,000 mg per day for most adults (19-50 years) (U.S. Department of Health and Human Services, 2021). Thus, the addition of calcium in GMY-gummy jelly would make it an alternative food source high in calcium.

The aim of this work was to develop a healthy gummy jelly product containing goat's milk yogurt, calcium, and collagen peptides with high nutritional values and antioxidant activity. The GMY-gummy jelly product qualities including pH, TA, total soluble solid, Brix/Acid ratio, color values, a_w , texture profile, chemical composition, ABTS

radical scavenging activity, ferric reducing antioxidant power, and metal chelating activity were also determined.

Materials and Methods

Materials

Fish collagen peptides powder (Bella colla collagen) was obtained from the Thai Nippon RABJ Co., Ltd. (Samut Sakhon, Thailand). Calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) was obtained from Mr. Chitchee (Samut Sakhon, Thailand). Goat's milk was obtained from the Sookjai Goat Milk Farm (Nakhon Ratchasima, Thailand). 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2, 4, 6-93 tripyridyl-s-triazine (TPTZ), and 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of Goat's Milk Yogurt

A thousand milliliter of whole goat milk was pasteurized at a temperature of 85°C for 15 min and cooled to 42-45°C. Then, the pasteurized goat milk was inoculated with 270 g of a commercial mixture starter culture yogurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and fermented at 42-45°C for 4-6 h. The final pH was 4.4 to 4.6. It was then rapidly cooled in a refrigerator and used for the preparation of goat's milk yogurt (GMY) gummy jelly.

Preparation of GMY-gummy Jelly Fortified with Calcium and Collagen

A preliminary sensory evaluation study, indicated the overall acceptability score of gummy jelly supplemented with 10% goat's milk yogurt was the highest. Therefore, 10% of goat's milk yogurt was incorporated in gummy jelly fortified with calcium and collagen peptides. The formulations of gummy jelly containing 10% goat's milk yogurt, 1% calcium hydroxyapatite, and various concentrations of collagen peptides (1, 1.5, and 2%) are shown in Table 1. In the preparation procedure, sugar, water, and glucose syrup were mixed, and boiled in a stainless-steel pot by a gas flame until a homogeneous mixture was obtained. Gelatin was mixed with water and heated in microwave until gelatin was transparent. Gelatin solution was then added into the previously prepared glucose syrup solution. When the temperature of the mixture was cooled to 70-80°C, the 10% (w/w) of goat's milk yogurt and citric acid powder were added and mixed. Calcium hydroxyapatite at concentration of 1% and various concentrations of collagen peptides (1, 1.5, and 2%)

were added and continued to mix. Some air bubbles were removed before the mixture was poured into a silicon mold and stored at 4°C for 12 h. After that, GMY-gummy jelly was unmolded and packed in a polypropylene (PP) bag for further experiments. T1, T2, and T3 referred to the GMY gummy jelly fortified with 1% calcium and 1, 1.5 or 2% of collagen, respectively. Control was GMY-gummy jelly without the addition of calcium and collagen. All samples were used for further experiments.

Table 1. The composition of goat's milk yogurt (GMY) gummy jelly fortified with 1% calcium (w/w) and various levels of collagen

Composition	Content % (w/w)			
	Control	T1	T2	T3
Gelatin (240 bloom)	7.0	7.0	7.0	7.0
Water	26.0	26.0	26.0	26.0
Sugar	30.0	28.0	27.5	27.0
Glucose syrup	25.0	25.0	25.0	25.0
Goat's milk yogurt	10.0	10.0	10.0	10.0
Citric acid	1.2	1.2	1.2	1.2
Sodium citrate	0.8	0.8	0.8	0.8
Collagen peptides	0.0	1.0	1.5	2.0
Calcium hydroxyapatite	0.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0

Control was GMY-gummy jelly without the addition of calcium and collagen; T1 was GMY-gummy jelly fortified with 1% calcium and 1% collagen; T2 was GMY-gummy jelly fortified with 1% calcium and 1.5% collagen; T3 was GMY-gummy jelly fortified with 1% calcium and 2% collagen.

Chemical and Physical Properties of GMY-gummy Jelly Fortified with Calcium and Collagen

Chemical Compositions Analysis

Moisture, protein, and ash contents of all samples were determined according to the method of AOAC (2000).

The pH, Total Titratable Acidity (TA), and Total Soluble Solid (TSS) Measurements

Five grams of control and T1, T2, and T3 were mixed with 25 mL of deionized water, homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany), and then centrifuged (HERMLE Labortechnik GmbH, Wehingen, Germany) at 3,500 rpm for 5 min. The supernatants were collected for pH, TA, TSS, and antioxidant activity measurements. The pH was measured using digital pH meter. The TA values were determined by titrating supernatants with 0.1 N NaOH, and calculated as percentage of citric acid equivalent. The TSS content was determined by hand refractometer.

Water Activity (a_w) Determination

Samples (Control, T1, T2, and T3) were cut into a small pieces, packed in a plastic sample box, and the water activity was measured by water activity meter (AQUA LAB 4TE dew point, Pullman, WA, USA).

Color Measurement

Color values of GMY-gummy jellies (Control, T1, T2, and T3) were measured in terms of CIE L*, a*, b* values using a color measurement device (Hunter Lab, Color flex 4510, USA).

Texture Evaluation

Texture properties of GMY-gummy jellies (Control, T1, T2, and T3) were evaluated using texture analyzer (TA-TX plus model, Stable Microsystems, Godalming, UK). The experiments conducted with texture profile analysis (TPA) mode using a TA-10 probe and test speed at 1 mm/s. Hardness, cohesiveness, springiness, and gumminess of the products were measured.

Evaluation of Antioxidant Activities

ABTS radical scavenging activity and ferric reducing antioxidant power (FRAP) of GMY-gummy jellies were done according to the methods of Wiriayaphan *et al.* (2012) and Benzie and Strain (1996), respectively. Results were calculated as mg Trolox equivalents (eq.)/100g sample. Fe²⁺-chelating activity was measured according to Decker and Welch (1990). Results were calculated as mg EDTA equivalents (eq.)/100 g sample.

Sensory Evaluation

Sensory evaluation of GMY-gummy jellies (Control, T1, T2, and T3) was performed by 42 untrained panelists using a preference test with 9-point hedonic scale (1=dislike extremely, 9=like extremely) to evaluate acceptability of appearance, color, flavor, texture, and overall acceptability. All panelists declared no food allergies. The GMY-gummy jelly treatment with high acceptability was selected for microbiological quality, consumer and purchase intention tests.

Microbiological Quality Test

GMY-gummy jelly fortified with 1% calcium and 2% collagen (T3) was tested for microbiological qualities (Total plate count, Fecal Coliform, *Escherichia coli* and *Staphylococcus aureus*) following the United States Food and Drug Administration (U.S. FDA) (1998) Biological Analytical Manual (BAM).

Consumer Test and Purchase Intention Test

A consumer panel of 300 volunteers recruited from the Rajamangala University of Technology Isan evaluated the GMY-gummy jellies using a preference test with 9-point hedonic scale (1=dislike extremely, 9=like extremely) to assess acceptability of appearance, color, flavor, texture, and overall acceptability. All panelists declared no food allergies. Purchase intention test was performed by using the question “Would you buy it?” accompanied by a structured scale of 4 points (“No”, “Yes”, “Maybe buy”, and “Maybe not buy”).

Statistical Analyses

All experiments were performed in triplicate. Data were expressed as means $\pm$ standard deviations and analyzed using analysis of variance (ANOVA); means comparison was done using Duncan's multiple range test (DMRT), at 95% confidence level.

Results and Discussion

Chemical and Physical Properties of GMY-gummy Jelly Fortified with Calcium and Collagen

Chemical Composition

The chemical composition of GMY-gummy jelly fortified with 1% calcium and various levels of collagen peptides are presented in Table 2. Moisture content is often measured in confectionary factories to control the degree of hydration, the rheological properties of products, and is an important factor governing texture, food quality, preservation, and resistance to deterioration (Nielsen, 2010; Delgado and Bañón, 2015). As shown in Table 2, the moisture content of T2 was the highest at 32.23%, while the lowest moisture content was found in T3 (29.48%). The lower moisture content of T3 might have been contributed by higher solid content than those of others. In general, moisture content of gummy confections ranged from 21.0 to 22.72% depending on the differences in formula, ingredients, processes, and drying temperature (Delgado and Bañón, 2015; Rivero *et al.*, 2020; Takeungwongtrakul *et al.*, 2020). The moisture contents obtained in the developed GMY-gummy jelly products were slightly higher than the ones from previous reports (Delgado and Bañón, 2015; Rivero *et al.*, 2020; Takeungwongtrakul *et al.*, 2020).

Ash content indicate the minerals content in food. The ash content of GMY-gummy jelly fortified with 1% calcium and 1-2% collagen (1.51-1.73%) was higher than control (0.78%) due to the

addition of calcium hydroxyapatite (Table 2). The ash content of reduced sugar strawberry (RSS) gummy jelly were 0.16-0.18% (Takeungwongtrakul *et al.*, 2020) while the ash content of gummy candy with pineapple juice and carrot juice were 0.46-0.48% (Achumi *et al.*, 2018). The ash content obtained in control and GMY-gummy jelly fortified with calcium and collagen were higher than the ones previously reported (Achumi *et al.*, 2018; Takeungwongtrakul *et al.*, 2020). Our results suggested that the developed GMY-gummy jelly products are a rich source of minerals.

The protein content of gummy jellies tended to increase with an increase in collagen peptides levels. T3 and T2 contained the highest protein content which were 10.19 and 9.96%, respectively (Table 2). Takeungwongtrakul *et al.* (2020) reported that protein content of the RSS-gummy jelly ranged to 8.35-8.47%. The protein content obtained in GMY-gummy jellies (T1-T3) were higher than the ones from RSS-gummy jelly. The differences in chemical composition of gummy jelly products could be due to differences in formula, ingredients, processing, and drying temperature. Our results suggested that the developed GMY-gummy jellies are a rich source of protein and minerals.

TSS, pH, TA, and Brix/TA ratio

TSS (Brix or g/100 g sample), pH titratable acidity (TA), and Brix/TA ratio are widely accepted as broad indicators of fruit flavor, and food product quality (Antunes, 2010). As shown in Table 2, a high TSS which ranged from 54.69-57.87 g/100 g sample was observed in all GMY-gummy jellies because of the high total sugar and glucose syrup content. There were no significant differences in TSS of GMY-gummy jellies supplemented with 1% calcium and 1-2% collagen (T1-T3) when compared with control ($p>0.05$), however, TSS of T1 was significantly lower than T3 ($p\leq 0.05$) (Table 2). TSS states the amounts of soluble solids in liquid which is dominated by total sugar content and a small portion of soluble proteins, amino acids and other organic materials (Bexiga *et al.*, 2017). All GMY-gummy jellies contained different levels of sugar whereby the control had the highest sugar content followed by T1, T2, and T3, respectively (Table 1). T3 contained the highest collagen peptides content followed by T2, T1, and control (without the addition of collagen and calcium), respectively. Therefore, soluble peptides and/or amino acids in collagen peptides might have contributed to the retention of TSS in T1-T3, resulting in no significant difference in the TSS of T1-T3 when compared with control. In addition, Basu *et al.* (2013) reported that prolonged thermal treatment initiates several physicochemical reactions,

including Maillard browning reaction. Therefore, TSS of T1 was significantly lower than that of T3 and that might have been due to formed insoluble sugars-peptides complexes by Maillard reaction. Charoen *et al.* (2015) reported that the addition of the crude extract of *Psidium guajava* leaf into the gummy jelly candy tended to increase the TSS content of products which were 64.0-75.0%. The average TSS content of gummy confections ranged from 66.33-77.7 Brix depending on drying time and different sugars (Delgado and Bañón, 2015; Curi *et al.*, 2017). The TSS of GMY-gummy jellies obtained in our experiment was lower than ones in previous reports by Charoen *et al.*, Curi *et al.*, and Delgado and Bañón due to high moisture content in GMY-gummy jellies.

The pH values of T1-T3 (4.41- 4.43) were significantly higher than control (4.18) due to alkalinity of the added calcium hydroxyapatite (Table 2). The pH of gummy jelly was found to be 2.8-3.1 (Jiamjariyatam, 2018). However, Delgado and Bañón (2015) reported that the average pH remained constant at 3.07 from 12 to 16 h and then increased to 3.10 and 3.12 at 20 h of drying time, while the pH of physalis jellies obtained from different sugars ranged from 3.20 to 4.07 (Curi *et al.*, 2017).

The TA values of GMY-gummy jellies were 0.72-0.76% (as citric acid) (Table 2). The TA values were not significantly different between T1, T2, and control, except for T3 which was slightly higher than control due to lower moisture content in T3. However, Brix/ acid ratio were not significantly different among all samples which were 74.17-76.72 (Table 2). The total acidity of physalis jellies obtained from different sugars varied from 0.09 to 0.13 g citric acid/100 g (Curi *et al.*, 2017), while

the TA values of the RSS-gummy jellies were 0.13-0.15% (Takeungwongtrakul *et al.*, 2020). The TA values of gummy candy with various levels of pineapple juice and carrot juice were 0.18-0.21 (Achumi *et al.*, 2018). TA values measure the total acid concentration in foods and is determined by titration of intrinsic acids with a standard base (Tyl and Sadler, 2017). Therefore, higher TA values observed in GMY-gummy jellies than the ones in previous reports by Achumi *et al.*, Curi *et al.*, and Takeungwongtrakul *et al.* could be due to the presence of lactic acid which is the main organic acid in yogurt samples.

Water Activity (a_w)

a_w is considered to be the most critical factor for microbial growth, determining quality, preservation, and shelf life of foodstuffs. There was no difference in a_w among all GMY-gummy jellies which ranged from 0.80-0.83 (Table 2), which is classified as intermediate moisture foods (IMF, $0.6 < a_w < 0.85$). These results indicated that the addition of 1% calcium and collagen at a concentration of 1-2% did not affect the a_w of products. Due to lower sugar content of T1-T3 than control, therefore, calcium and collagen peptide additions might have contributed to the retention of a_w in T1-T3, resulting in no difference in a_w among all GMY-gummy jellies (Table 1). Charoen *et al.* (2015) also reported that the addition of different levels of crude extract of *Psidium guajava* leaf into gummy jelly had no impact on the water activity of the products which were 0.75-0.79. The RSS-gummy jellies contained a_w of 0.79- 0.72 (Takeungwongtrakul *et al.*, 2020). The a_w of gummy confections ranged from 0.72 to 0.71 at 12-16 h to 20 h of drying times (Delgado and Bañón, 2015).

Table 2. Chemical and physical properties of GMY-gummy jelly fortified with 1% calcium and various levels of collagen

Chemical and physical properties	Control	T1	T2	T3
Moisture content (%w.b.)	30.20±0.24 ^b	30.41±0.27 ^b	32.23±0.29 ^a	29.48±0.43 ^c
Ash content (%w.b.)	0.78±0.02 ^b	1.51±0.39 ^a	1.73±0.03 ^a	1.60±0.04 ^a
Protein content (%w.b.)	8.39±0.15 ^c	9.61±0.15 ^b	9.96±0.11 ^a	10.19±0.12 ^a
TSS (g/100 g sample)	55.49±2.36 ^{ab}	54.69±1.63 ^b	55.38±0.79 ^{ab}	57.87±0.42 ^a
pH	4.18±0.06 ^b	4.43±0.01 ^a	4.41±0.00 ^a	4.43±0.01 ^a
TA (%) (as citric acid)	0.72±0.01 ^b	0.74±0.02 ^{ab}	0.73±0.00 ^{ab}	0.76±0.03 ^a
Brix/TA ratio ^{ns}	76.72±1.84	74.14±2.99	76.08±1.35	76.06±3.86
a_w ^{ns}	0.8172±0.01	0.8180±0.01	0.8307±0.02	0.8083±0.02
Color				
L^*	63.69±0.29 ^d	67.41±0.18 ^c	68.14±0.41 ^b	68.18±0.38 ^a
a^*	2.79±0.01 ^d	3.29±0.02 ^b	3.06±0.06 ^c	3.69±0.04 ^a
b^*	5.42±0.16 ^d	7.58±0.24 ^b	6.86±0.40 ^c	8.27±0.05 ^a

Different superscripts within a row indicate significant differences ($p \leq 0.05$); ns means not significantly different in the same row ($p > 0.05$); Control was GMY-gummy jelly without the addition of calcium and collagen; T1 was GMY-gummy jelly fortified with 1% calcium and 1% collagen; T2 was GMY-gummy jelly fortified with 1% calcium and 1.5% collagen; T3 was GMY-gummy jelly fortified with 1% calcium and 2% collagen

However, a lower a_w was observed in the gummy jelly containing gelatin content (9, 12, and 15%) and isomaltulose content (20, 30, 40, 50, and 100%) which were 0.5- 0.6 (Jiamjariyatam, 2018). The difference in a_w of gummy jelly products could be due to differences in formula, ingredients, processing and drying temperature.

Color Values

The addition of collagen tended to increase L^* (lightness), a^* (redness), and b^* (yellowness) values of all GMY-gummy jellies when compared to the control (Table 2). L^* , a^* , and b^* values of T3 were the highest at 68.18, 3.69, and 8.27, respectively. That is, T3 was the clearest jelly formulation and with greater red and yellow intensity coloration. These results indicated that calcium and collagen had a direct effect on color of the GMY-gummy jelly due to the white color of calcium and collagen powder.

Texture Values

Hardness, cohesiveness, and springiness were not significantly different among all GMY-gummy jellies ($p > 0.05$, Table 3). These results indicated that the addition of calcium and collagen did not affect the hardness, cohesiveness, and springiness of gummy jelly products when compared to control. However, the gumminess of gummy jelly supplemented with 1.5% collagen (0.19 kg/s) was slightly lower than control (0.23 kg/s), but was not significantly different from 1 and 2% collagen (Table 3). T2 contained a higher moisture content

than the others and this might have contributed to the lower gumminess.

Sensory Evaluation

Sensory attributes: appearance, color, flavor, texture, and overall acceptability of gummy jelly products are shown in Table 4. Color and flavor attributes score of T1-T3 were higher than control. The appearance and overall acceptability attributes score of control were lower than T1 and T3, but were not significantly different with T2. No significant differences of texture score were detected among GMY-gummy jellies. These results implied that the addition of calcium and collagen improved all sensory attributes score of the GMY-gummy jellies, except for texture. Overall acceptability score of GMY-gummy jellies with 1% calcium and 1-2% collagen ranged from 7.12-7.33 which revealed the like moderately score (Table 4).

Antioxidant Activities

ABTS radical scavenging activity, FRAP values, and metal chelating activity of control were 1.65 mg Trolox equivalents/100 g sample, 11.58 mg Trolox equivalents/100 g sample, and 35.30 mg EDTA equivalents/100 g sample, respectively (Figure 1a and Figure 1b). The presence of different bioactive peptides and free amino acids derived from milk proteins through proteolysis by LAB were related to antioxidant capacity of plain yogurt (Perna *et al.*, 2014). In addition, gelatin and gelatin hydrolysates exhibited antioxidant capacity by FRAP, ABTS, and metal chelating assays (Giménez

Table 3. Texture of GMY-gummy jelly fortified with 1% calcium and various levels of collagen

Treatments	Hardness ^{ns} (kg/s)	Cohesiveness ^{ns}	Springiness ^{ns} (mm/s)	Gumminess (kg/s)
Control	0.23±0.01	0.96±0.03	3.82±0.03	0.23±0.02 ^a
T1	0.23±0.00	0.94±0.02	3.86±0.87	0.22±0.00 ^{ab}
T2	0.20±0.01	0.94±0.01	3.77±0.05	0.19±0.00 ^b
T3	0.22±0.03	0.93±0.03	3.80±0.08	0.20±0.03 ^{ab}

Different superscripts within a column indicate significant differences ($p \leq 0.05$); ns means not significantly different in the same column ($p > 0.05$); Control was GMY-gummy jelly without the addition of calcium and collagen; T1 was GMY-gummy jelly fortified with 1% calcium and 1% collagen; T2 was GMY-gummy jelly fortified with 1% calcium and 1.5% collagen; T3 was GMY-gummy jelly fortified with 1% calcium and 2% collagen

Table 4. Sensory evaluation of GMY-gummy jelly fortified with 1% calcium and various levels of collagen

Treatments	Appearance	Color	Flavor	Texture ^{ns}	Overall acceptability
Control	6.81±1.22 ^b	6.05±1.36 ^b	6.31±1.60 ^b	7.02±1.30	6.33±0.71 ^b
T1	7.24±1.09 ^a	6.31±1.42 ^a	7.10±1.11 ^a	7.17±1.21	7.33±0.99 ^a
T2	6.90±1.06 ^{ab}	6.29±1.35 ^a	6.67±1.30 ^a	7.02±1.28	7.12±1.14 ^{ab}
T3	7.07±1.12 ^a	6.43±1.48 ^a	6.86±1.47 ^a	7.14±1.12	7.31±1.24 ^a

Different superscripts within a column indicate significant differences ($p \leq 0.05$); ns means not significantly different in the same column ($p > 0.05$); Control was GMY-gummy jelly without the addition of calcium and collagen; T1 was GMY-gummy jelly fortified with 1% calcium and 1% collagen; T2 was GMY-gummy jelly fortified with 1% calcium and 1.5% collagen; T3 was GMY-gummy jelly fortified with 1% calcium and 2% collagen

et al., 2009). Therefore, bioactive peptides obtained from the proteolytic activity of milk proteins by LAB and added gelatin could have contributed to the antioxidant activities of control GMY-gummy jelly. The antioxidant activities of T1-T3 were higher than control (Figure 1a and Figure 1b). These results indicated that collagen peptides were related to increased antioxidant activity of the GMY-gummy jelly. Fish collagen contains a large number of antioxidant amino acids residues, such as Val, Met, Trp, Tyr, Arg, Glu, Asp, and Phe (Ding *et al.*, 2019; Wang *et al.*, 2020). Therefore, those amino acid residues in collagen peptides might relate to the antioxidant activities of GMY-gummy jelly. Our results suggested that the developed GMY-gummy jelly supplemented with collagen is a potential good source of antioxidant substances. Based on

the antioxidant activity assays and sensory evaluation, the gummy jelly supplemented with 1% calcium and 2% collagen (T3) was selected for microbiological qualities analysis, consumer, and purchase tests.

Microbiological Qualities

Microbiological qualities of gummy jelly product supplemented with 1% calcium and 2% collagen (T3) are shown in Table 5. Total plate count of T3 was 2.3×10^3 CFU/g of sample, while yeasts and molds, *E. coli* and *S. aureus* were not detected. These results indicated that microbiological qualities of T3 did not exceed the limitation of Thai community product standard (Frozen Dried Jelly, 520/2547) (2004) (Table 5).

Table 5. Microbiological qualities of GMY- gummy jelly fortified with 1% calcium and 2% collagen (T3)

Microbiological Test	Microbiological qualities	
	T3	Thai community product standard, Frozen Dried Jelly (No. 520/2547)
Total Plate Count	2.3×10^3 CFU/g	$< 1 \times 10^4$ CFU/g
Yeasts and Molds	Not detected in 1 g	< 100 CFU/g
<i>E. coli</i>	Not detected in 1 g	< 3 MPN /g
<i>S. aureus</i>	Not detected in 1 g	Not detected in 1 g

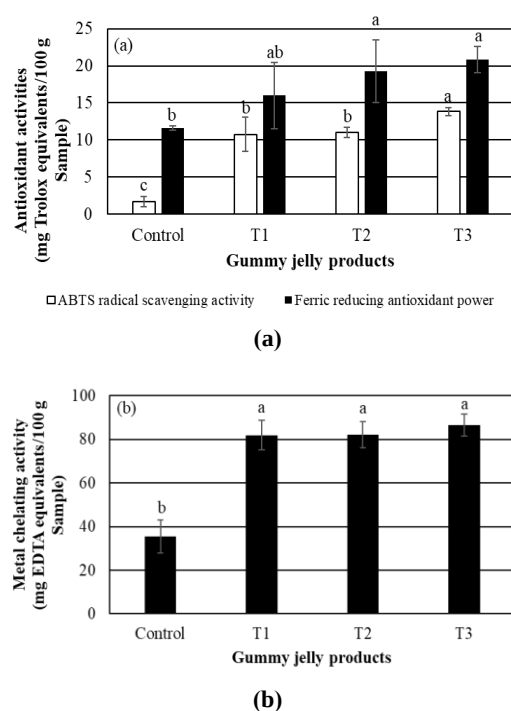


Figure 1. Antioxidant activity of goat's milk yogurt (GMY) gummy jelly fortified with 1% calcium and various levels of collagen; (a) ABTS radical scavenging activity and Ferric reducing antioxidant power; (b) Metal chelating activity. Bars with different letters indicate means with significant differences ($p \leq 0.05$); Control was GMY-gummy jelly without the addition of calcium and collagen; T1 was GMY- gummy jelly fortified with 1% calcium and 1% collagen; T2 was GMY-gummy jelly fortified with 1% calcium and 1.5% collagen; T3 was GMY-gummy jelly fortified with 1% calcium and 2% collagen

Consumer and Purchase Tests

The consumer's panel consisted of 300 people, 163 people were male and 137 people were female, mostly aged between 17 and 25 years. The panelists were recruited among the staff and student population of the Rajamangala University of Technology Isan. The sensory attributes: appearance, color, flavor, texture, and overall acceptability of T3 are shown in Table 6. All attributes score of gummy jelly products supplemented with 1% calcium and 2% collagen ranged from 7.62-7.87 (Like Moderately to Like Very Much). The results obtained through the purchase test exhibited that 53.33% of the volunteers answered "I would buy it"; while only 1.67% answered "I would not buy it"; 37.67% of the volunteers responded "maybe buy it"; and 7.33% of the volunteers responded "maybe not buy it" to the poll (data not shown). These results indicated that the GMY-gummy jelly supplemented with 1% calcium and 2% collagen was generally well accepted by consumers.

Table 6. Sensory evaluation of GMY- gummy jelly fortified with 1% calcium and 2% collagen (T3)

Sensory attributes	Score (Means $\pm$ SD)	Description
Appearance	7.85 $\pm$ 0.90	Like Moderately to Like Very Much
Color	7.79 $\pm$ 1.03	Like Moderately to Like Very Much
Flavor	7.62 $\pm$ 0.98	Like Moderately to Like Very Much
Texture	7.73 $\pm$ 0.92	Like Moderately to Like Very Much
Overall acceptability	7.87 $\pm$ 0.90	Like Moderately to Like Very Much

N = 300

Conclusions

The addition of 1% calcium and collagen at levels of 1-2% tended to increase product qualities including pH, color values, antioxidant activities, ash content, protein content, and all sensory attributes score, however, it did not affect the TA, TSS, texture, a_w , and Brix/Acid ratio of GMY gummy jellies when compared to control. Based on the antioxidant activities and protein content, the optimum level of collagen supplemented in GMY-gummy jelly was 2%. The developed GMY-gummy jelly product supplemented with 1% calcium and 2% collagen was accepted by 300 people with 7.62-7.87 score. The purchase test exhibited that 53.33% of the volunteers would buy the developed GMY-gummy jellies. In addition, the microbiological qualities of the product did not exceed the limitation of Thai community product standard (520/2547). Our findings indicated that the developed goat's milk yogurt gummy jelly with 1% calcium and 2% collagen is potentially a good source of protein, minerals and antioxidant substances.

Acknowledgements

The authors would like to thank Rajamangala University of Technology Isan for facilities supports. We would also like to thank Ms. Martha Maloi Eromine for her support in editing the English language in this manuscript.

References

Achumi, L. V., Shanta Peter, E. R., and Das, A. (2018). Studies on preparation of gummy candy using pineapple juice and carrot juice. *Int. J. Chem. Stud.*, 6(5):1,015-1,018.

- Antunes, L.E.C., Ristow, N.C., Krolow, A.C., Carpenedo, S., and Júnior, C. R. (2010). Yield and quality of strawberry cultivars. *Hortic. Bras.*, 28(2):222-226.
- AOAC. (2000). Official Methods of Analysis. 17 th Edition. The Association of Official Analytical Chemists, Gaithersburg, MD, USA.
- Basu, S., Shivhare, U.S., Singh, T.V. (2013). Effect of substitution of stevioside and sucralose on rheological, spectral, color and microstructural characteristics of mango jam. *J. Food Eng.*, 114(4):465-476.
- Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. *Anal. Biochem.*, 239(1):70-76.
- Bexiga, F., Rodrigues, D., Guerra, R., Brázio, A., Balegas, T., Cavaco, A. M., Antunes, M. D., and de Oliveira, J. V. (2017). A TSS classification study of 'Rocha' pear (*Pyrus communis* L.) based on noninvasive visible/near infra-red reflectance spectra. *Postharvest Biol. Technol.*, 132:23-30.
- Burey, P., Bhandari, B., Rutgers, R., Halley, P., and Torley, P. (2009). Confectionery gels: A review on formulation, rheological and structural aspects. *Int. J. Food Prop.*, 12(1):176-210.
- Charoen, R., Savedboworn, W., Phuditcharnchnakun, S., and Khuntaweetap, T. (2015). Development of antioxidant gummy jelly candy supplemented with *Psidium guajava* leaf extract. *KMUTNB Int. J. Appl. Sci.*, 8(2):145-151.
- Čižauskaitė, U., Jakubaitytė, G., Žitkevičius, V., and Kasparavičienė, G. (2019). Natural ingredients-based gummy bear composition designed according to texture analysis and sensory evaluation *in vivo*. *Molecules*, 24(7):1,442.
- Curi, P.N., Carvalho, C. dos S., Salgado, D.L., Pio, R., Pasqual, M., Souza, F.B.M. de, and Souza, V. R. de. (2017). Influence of different types of sugars in physalis jellies. *Food Sci. Technol.*, 37(3):349-355.
- De Santis, D., Giacinti, G., Chemello, G., and Frangipane, M. T. (2019). Improvement of the sensory characteristics of goat milk yogurt. *Food Sci. Technol.*, 84(8):2,289-2,296.
- Decker, E. A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.*, 38(3):674-677.
- Delgado, P. and Bañón, S. (2015). Determining the minimum drying time of gummy confections based on their mechanical properties. *CYTA J. Food.*, 13(3):329-335.
- Ding, D., Du, B., Zhang, C., Zaman, F., and Huang, Y. (2019). Isolation and identification of an antioxidant collagen peptide from skipjack tuna (*Katsuwonus pelamis*) bone. *RSC Adv.*, 9(46):27,032-27,041.
- Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., and Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *J. Funct. Foods.*, 48:387-399.
- Giménez, B., Alemán, A., Montero, P., and Gómez-Guillén, M. C. (2009). Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. *Food Chem.*, 114(3):976-983.
- Hashim, I. B., Khalil, A.H., and Afifi, H.S. (2009). Quality characteristics and consumer acceptance of yogurt fortified with date fiber. *J. Dairy Sci.*, 92(11):5,403-5,407.
- Jiamjariyatam, R. (2018). Influence of gelatin and isomaltulose on gummy jelly properties. *Int. Food Res. J.*, 25(2):776-783.
- Lele, V., Ruzauskas, M., Zavistanaviciute, P., Laurusiene, R., Rimene, G., Kiudulaite, D., Tomkeviciute, J., Nemeikstyte, J., Stankevicius, R., and Bartkiene, E. (2018). Development and characterization of the gummy-supplements, enriched with probiotics and prebiotics. *CYTA J. Food.*, 16(1):580-587.

- Nielsen, S.S. (2010). Determination of moisture content. In: Food Analysis Laboratory Manual, Food Science Texts Series. Nielsen, S.S. (ed.). Springer, MA, p. 17-27.
- Nurilmala, M., Hizbullah, H.H., Karnia, E., Kusumaningtyas, E., and Ochiai, Y. (2020). Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from Yellowfin Tuna (*Thunnus albacares*) skin. *Mar. Drugs.*, 18(2):98.
- O'Keefe, J.H., Bergman, N., Carrera-Bastos, P., Fontes-Villalba, M., DiNicolantonio, J.J., and Cordain, L. (2016). Nutritional strategies for skeletal and cardiovascular health: hard bones, soft arteries, rather than vice versa. *Open Heart.*, 3(1):e000325.
- Perna, A., Intaglietta, I., Simonetti, A., and Gambacorta, E. (2014). Antioxidant activity of yogurt made from milk characterized by different casein haplotypes and fortified with chestnut and sulla honeys. *J. Dairy Sci.*, 97(11):6,662-6,670.
- Rivero, R., Archaina, D., Sosa, N., Leiva, G., Baldi Coronel, B., and Schebor, C. (2020). Development of healthy gummy jellies containing honey and propolis. *J. Sci. Food Agric.*, 100:1,030-1,037.
- Takeungwongtrakul, S., Thavarang, P., and Sai-Ut, S. (2020). Development of Strawberry gummy jelly with reduced sugar content from Strawberry syrup. *Int. J. Agric. Technol.*, 16(5):1,267-1,276.
- Thai Community Product Standards, Frozen Dried Jelly (No. 520/2547). (2004). Frozen Dried Jelly. Bangkok: Thailand. Available from: http://tcps.tisi.go.th/pub/tcps520_47.pdf. Accessed date: Jun 27, 2021.
- Tyl, C., and Sadler, G.D. (2017). pH and titratable acidity. In: Food Analysis. Nielsen, S.S. (ed). Springer, Switzerland, p. 389-406.
- U. S. Department of Health and Human Services, National Institutes of Health. (2021). Calcium Fact Sheet for Consumers. Maryland: USA. Available from: <https://ods.od.nih.gov/factsheets/Calcium-Consumer/#:~:text=Calcium%20is%20a%20mineral%20found,supports%20their%20structure%20and%20hardnes>. Accessed date: Jun 27, 2021.
- United States Food and Drug Administration (U.S. FDA). (1998). Biological Analytical Manual (BAM). Virginia: USA. Available from: <https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam>. Accessed date: June 27, 2021.
- Verruk, S., Dantas, A., and Prudencio, E.S. (2019). Functionally of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *J. Funct. Foods.*, 52:243-257.
- Wang, W. Y., Zhao, Y. Q., Zhao, G. Z., Chi, C. F., and Wang, B. (2020). Antioxidant peptides from collagen hydrolysate of Redlip Croaker (*Pseudosciaena polyactis*) scales: preparation, characterization, and cytoprotective effects on H₂O₂-damaged HepG2 cells. *Mar. Drugs.*, 18(3):156.
- Wiriayaphan, C., Chitsomboon, B., and Yongsawadigul, J. (2012). Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chem.*, 132(1):104-111.