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Formulation of Gelatin-Based Wheatgrass Leaf Juice Gummy Jellies with Antioxidant and the Analyses of Physicochemical and Texture Properties as Well as Evaluate the Nutritional Property of Selected Formulation

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Abstract

The aim of this study was to develop gummy jellies from wheatgrass leaf juice, assess the antioxidant activity of wheatgrass, and study the effects of gelatin (10, 20, 30 and 40% of honey weight) on the physicochemical properties and texture using different techniques (warm and hot), as well as evaluate the nutritional property of selected formulation. The antioxidant activity of wheatgrass was determined using the DPPH assay. The 50% effective concentration (EC_{50}) was 23.42±1.13 mg sample/mL. This concentration was then used in formulation of gummy jellies. The samples were investigated for moisture, water activity, pH, color, and texture. The moisture, water activity and pH were in the range of 0.66±0.07 - 6.20±0.62 %, 0.72±0.03 - 0.87±0.02 and 4.76±0.08 - 5.92±0.04, respectively. The result suggested that gummy jellies had low moisture, water activity and pH, particularly the 10% honey weight of gelatin concentration using the hot technique (H1 formulation). These low values contribute to potentially increase in their shelf life. The gummy jellies were red-yellowish in color. The texture of the H1 formulation was not significantly different ($p>0.05$) in hardness, gumminess and chewiness compared to 20-30% honey weight of gelatin using both hot and warm techniques. Therefore, the H1 formulation was selected for the analysis of nutritional properties. The energy of the H1 formulation was 56 kcal/50 g per one serving. Most of the energy of the gummy jelly came from sugar (18 g). According to the recommendation by WHO, adults and children should consume less than 25 grams of sugar per day to reduce the risk of NCDs. Therefore, the gummy jelly is suitable for less than 2 servings per day.

Introduction

Gummy jelly is a confectionery product in a group of candy gel. Commercial gummy jellies are products based on sugar, gelling agent and food additives (Cedeno-Pinos et al., 2021). The commonly used gelling agents are gelatin, pectin and starch (Takeungwongtrakul et al., 2020). Gelatin is used as a main gelling agent that provides structure in the gummy jellies (Mutlu et al., 2018). The popular sweetener sources are glucose,

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sucrose, syrups which have a high glycemic index and contribute to high blood sugar level (Jiamjariyatam, 2018). Long-term high blood glucose levels may induce obesity, cardiovascular disease, diabetes and other diseases, which are all serious threats to human health (Gan et al., 2022). Reducing or replacing sugars with honey might represent healthier alternatives. Honey is a functional food produced by bees (*Apis mellifera*) which contains a wide variety of bioactive substances and enzymes giving it antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic properties (Mutlu et al., 2018). Replacing sucrose with honey also decreases the high glycemic index of the product (Rivero et al., 2020). Food additives of gummy jelly product also have negative effects on health. In the past years, several fruit, vegetable and herb have been considered for the development of functional gummy jellies (Teixeira-Lemos et al., 2021; Takeungwongtrakul et al., 2020; Charoen et al., 2015) such as gummy jelly mixed with *Thunbergia laurifolia* Linn. extract aimed to reducing breath alcohol content (Kitpot et al., 2020). Therefore, an addition of healthier ingredients in gummy jelly may be less detrimental to health by the product.

Wheatgrass (*Triticum aestivum* Linn.) belongs to the family Poaceae. The juice of wheatgrass is known as "green blood" because it contains a large amount of chlorophyll, making up 70% of the total chemical constituents (Choudhary et al., 2021). Chlorophyll was shown to have positive effects on thalassemia patients undergoing blood transfusion and reduce toxicity in cancer patients (Ove et al., 2021). Wheatgrass juice is also an abundant source of essential vitamins and minerals such as retinol, ascorbic acid, tocopherol, vitamin B complex, calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron and molybdenum (Adhikari et al., 2022). Clinically, wheatgrass juice is mainly utilized because of its antioxidant property. It is reported that wheatgrass contain antioxidant compounds such as carotenoids, tocopherols, tocotrienols, phenolic acids, phytic acid, phytosterols and flavonoids (Kaur et al., 2021). Furthermore, it is a significant herbal plant used to treat various diseases and disorders such as high blood pressure, obesity, cancer, diabetes, kidney swelling, gastritis, ulcers, astriction, anemia, eczema, pancreas, diuresis, fatigue, anemia, asthma, hemorrhoids, halitosis, skin problem and constipation (Choudhary et al., 2021; Jiang et al., 2021).

This study aimed to develop gummy jellies from wheatgrass juice containing antioxidants and investigate effects of gelatin content (10, 20, 30 and 40% of honey weight) on the physicochemical properties and texture using different techniques (warm and hot) as well as evaluate nutritional properties of selected formulation. The results would be useful as a guide to further develop commercial products that are also healthier alternatives.

Materials and methods

1. Preparation of wheatgrass leaf juice

Fresh wheatgrass leaves were obtained locally from Namai, Ladlumkaew, Pathumthani Province, Thailand. Wheatgrass leaves (60 g) were cleaned before squeezing with a juicer MC-911-1 (Jyu Fong Machinery Co., LTD, Taichung, Taiwan). The sample was centrifuged at 4500 rpm for 10 min. The percent extract (% yield) was calculated using the formula below (Smith et al., 2015). Then, the antioxidant activity of the extract was measured and applied on healthy gummy jelly supplement.

$$\%\text{yield} = \frac{wheat\ grass\ juice}{initial\ weight\ of\ wheat\ grass\ leaves}\ x\ 100$$

2. The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay of wheat grass leaf juice

The antioxidant activity method was adapted from Sembiring et al. (Sembiring et al., 2018). Different concentrations of standard (ascorbic acid, 1-10 µg/mL) were prepared. One-hundred microliter of either standard (Sigma-Aldrich, St Louis, MO, U.S.A.) or sample (200 - 1000 mg sample/mL) solutions were mixed with 100 µL of 208 µM DPPH (Sigma-Aldrich, St Louis, MO, U.S.A.) in methanol (Merck, Darmstadt, Germany). After incubating in darkness for 30 min, the absorbance was measured at 517 nm using a microplate reader. All reactions were carried out in triplicate. Antioxidant capacity was expressed as the concentration of extract providing half maximal effective concentration (EC_{50}). The EC_{50} was determined graphically by plotting the percent inhibitions (%inhibition) against the extract concentrations. The percent inhibition was calculated using the following formula:

$$\%inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where: $A_{control}$ is the absorbance of the DPPH solution
A_{sample} is the absorbance of sample or standard with DPPH solution

3. Preparation of healthy gummy jelly

Gummy jelly was prepared using two techniques (Mutlu et al., 2018), warm (50-55°C) and hot (115°C) with four different gelatin doses (10, 20, 30 and 40% of honey weight). The formulations are shown in Table 1. Each gelatin (McGarett, JR F & B Co., Ltd., Bangkok, Thailand) dose was added to 100 mL of distilled water and kept for 5 min. Then, excess water was completely removed and the soaked gelatin was used as gelling agent. The soaked gelatin was dissolved at 70°C for 30 min in a water bath (Memmert, Schwabach, Germany). In the warm technique, dissolved gelatin was mixed with 50 g of honey (the Royal Chitralada Project, Bangkok, Thailand) and 23.42 mg/mL of wheatgrass juice. During mixing at 200 rpm for 1 min with a stirrer (Daihan Scientific, Gangwon-do, Korea), the mixtures were kept at 50-55°C. For the hot technique, the soaked gelatin was added into the 50 g of honey and 23.42 mg/mL of wheatgrass juice. The mixtures were manually stirred by a glass rod on a hot plate for 20 min, to ensure that the gelatin did not set before reaching the desired temperature. During this process, the temperature of mixture was kept at approximately 115°C. Mixtures from both techniques were poured into a bear shape silicone mold (width x length x thickness; 10 x 10 x 5 mm) and kept for 30 min in a fridge at 4°C. The jelly gummies were removed from the mold and kept for three days in a desiccator. After this process, the honey jelly gummies were placed in plastic bags and stored at 4°C.

Table 1 Formulations of healthy gummy jelly from wheatgrass juice

Formulations	Gelatin doses (g) (% of honey weight)	Wheatgrass juice (mL)	Honey (g)
Warm technique			
W1	5 (10)	0.64	50
W2	10 (20)	0.70	50
W3	15 (30)	0.77	50
W4	20 (40)	0.82	50
Hot technique			
H1	5 (10)	0.64	50
H2	10 (20)	0.70	50
H3	15 (30)	0.77	50
H4	20 (40)	0.82	50

4. Physicochemical analysis

The moisture content measurement was performed using a moisture analyzer MA37 (Sartorius, Goettingen, Germany). The condition was set to the fully automatic mode. Ten grams of gummy jelly sample was approximately weighed on the moisture analyzer. Water activity was measured by the auto start mode using water activity meter LabSwift-aw (Novasina, Lachen, Switzerland). The whole gummy jelly sample was added to a sample dish then put into the water activity meter. After the analysis, the actual values in water activity and moisture were recorded as a_w and % moisture, respectively. The measurements were carried out in triplicate.

The pH value was determined by homogenizing the sample (1 g) in distilled water (9 mL) at 50°C and measured using a digital pH meter (SI analytics, Mainz, Germany). The measurements were carried out in triplicate.

The color of gummy jelly was analyzed using Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan). The color meter was calibrated using a white plate CR-A43 and assessed using the DP mode. The measurement was carried out in triplicate. The analyzed color parameters were L*, a*, b* using the following implication: L* from (0) black to (100) white, a* from (-)greenness to (+) redness, b* from (-) blueness to (+) yellowness.

Texture analysis of gummy jelly was evaluated using texture analyzer CT3 (Ametek Brookfield, Middleborough, MA, U.S.A.) equipped with the cylindrical (35 mm.) probe with texture profile analysis (TPA) mode using the test speed of 1 mm/s. The hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were reported. The measurement was carried out in triplicate.

5. Nutritional analysis

The selected sample were analyzed for nutritional values (total energy, carbohydrate, protein and fat) using the nutritional analysis program (INMUCAL-Nutrients version 4.0, Institute of Nutrition, Mahidol University). The obtained data were reported as 50 g of gummy jelly sample (1 serving).

6. Data analysis

The experiment was performed using a completely randomized design. Statistical data were analyzed for variance using ANOVA (SPSS software, version 29). The LSD's test was applied to detect the differences among the samples. The mean difference is significant at the 0.05 level ($p \leq 0.05$).

Results and discussion

1. Antioxidant activity of wheatgrass leaf juice

The fresh wheatgrass leaves were processed by squeezing and gave the final yield of approximately 50 w/v. The antioxidant activity of wheatgrass juice at 50% effective concentration (EC_{50}) was 23.42±1.13 mg sample/mL. This concentration of the wheatgrass juice resulted in a 50% free radical (DPPH) inhibition. There are reports that free radical is abundant in the cell of the human body and can cause biological damage (Martemucci et al., 2022). Therefore, the 23 mg/mL of sample juice was added to gummy jelly.

2. Physicochemical properties

The moisture and water activity were in the range of 0.66±0.07-6.20±0.62 % and 0.72±0.03-0.87±0.02, respectively (Table 2). The increase in the moisture content and water activity of gummy jellies may be affected by the mixing technique and gelatin dose, as in formulations with higher gelatin content may not have been vaporized enough during the hot mixing. The moisture content of gummy jellies was lower than the general recommendation (less than 20%) for this type of product (Renaldi et al., 2022). Moreover, the moisture content of W1 (0.66±0.07) formulation was low and not significantly different ($p>0.05$) from the H1 (0.77±0.25) and H2 (1.30±0.65) formulations. Probably due to the gelatin content was not quite different, resulting in the moisture content of 3 formulations were not different. The water activity affects the growth of microorganisms which negatively correlates with the stability of the products. It is reported that the water activity in the range of 0.7 – 0.8 of a gummy jelly had a high stability (Periche et al., 2014). In particular, the W1 and H1 formulations had no significant difference in low water activity. Therefore, the low moisture content and water activity of W1 and H1 may enable good conservation.

The pH values of the gummy jellies are shown in Table 2. The pH values of gummy jellies ranged from 4.76±0.08-5.92±0.04. The pH values of gummy jellies were affected by the mixing technique and gelatin dose. The pH of the hot mixing technique decreased because the hexose was oxidized to acidic sugar at high temperature (Mutlu et al., 2018). The result showed that the increase in gelatin resulted in an increase of the pH value. This is because the gelatin was derived from collagen which is an amino acid and is known to have high pH value of gummy jellies (Jiamjariyatam, 2018). Periche et al. (2014) reported that less of pH could affect the increase in their shelf life. Specifically, the pH value of H2 formulation showed the lowest value but was not significantly different from that of the H1 and W1 formulas. The results suggested that H1, H2 and W1 formulations might have a longer shelf life compared to other formulations.

The L*, a* and b* values of the gummy jellies ranged from 27.23±0.67-32.12±1.74, 0.24±0.03-1.21±0.05 and 1.92±1.65-7.10±0.63, respectively (Table 2). The high gelatin content tends to increase brightness. The positive a* and b* values showed that the gummy jellies were red-yellowish in color. This result correlated with a study of Rivero et al. (2020) with a positive a* and b* value of the honey gummy jelly (Rivero et al., 2020). The yellow pigment of gummy jellies was probably derived from the phytochemical compounds in the honey such as rutin, caffeic acid, quercetin and kaempferol (Vazquez et al., 2021). These compounds were major groups of phenolic acid and flavonoids which are responsible for the antioxidant activity of honey (Hossen et al., 2017; Rubio-Arraez et al., 2016). The highest a* value was of the H1 (1.21±0.05) formulation. The b* value of W4 (7.10±0.63) formulation was the highest, followed by H1 (5.58±0.26) and H3 (5.18±0.26). The results indicated that H1 formulation was high in all a* and b* values.

Table 2 Moisture, water activity, pH, and color of gummy jellies

Formulas	properties					
	Moisture (%)	Water activity (a_w)	pH	Color		
				L*	a*	b*
W1	0.66±0.07^{e}	0.72±0.03^{c}	5.00±0.05^{c}	27.23±0.6^{7c}	0.30±0.08^{cd}	2.68±0.61^{d}
W2	2.03±0.37^{d}	0.79±0.01^{b}	5.70±0.07^{a}	32.12±1.74^{a}	0.68±0.83^{b}	3.58±0.15^{cd}
W3	2.84±0.27^{c}	0.85±0.05^{ab}	5.91±0.09^{a}	27.67±0.91^{c}	0.41±0.14^{cd}	2.72±0.30^{d}
W4	4.98±0.11^{b}	0.87±0.02^{a}	5.92±0.04^{a}	30.26±0.19^{b}	1.04±0.17^{a}	7.10±0.63^{a}
H1	0.77±0.25^{e}	0.76±0.04^{bc}	4.77±0.15^{cd}	29.86±0.32^{b}	1.21±0.05^{a}	5.58±0.26^{b}
H2	1.30±0.65^{e}	0.81±0.05^{b}	4.76±0.08^{cd}	30.90±0.04^{ab}	0.50±0.23^{bc}	2.55±0.44^{d}
H3	6.20±0.62^{a}	0.85±0.03^{a}	5.58±0.54^{ab}	31.19±0.15^{ab}	1.20±0.14^{a}	5.18±0.26^{b}
H4	4.80±0.52^{b}	0.86±0.04^{a}	5.22±0.19^{bc}	30.69±0.78^{b}	0.24±0.03^{d}	1.92±1.65^{de}

Remark: $^{a-e}$ different letters in the same column indicate values that are significantly different in statistics ($p\leq0.05$).

3. Texture properties

The texture profiles of gummy jellies including hardness (233.50±7.78-1020.50±44.55 g), adhesiveness (0.01±0.00-0.10±0.14 mJ), cohesiveness (0.82±0.32-0.95±0.16), springiness (4.66±0.15-4.80±0.15), gumminess (181.50±44.55-924.50±10.61 g) and chewiness (8.30±2.26-42.75±2.92 mJ) are shown in Table 3. Different gelatin contents and techniques had no significant impact on springiness, cohesiveness as well as adhesiveness properties (p>0.05). However, the texture indicators that are particularly relevant to gelled confections are hardness, chewiness and gumminess, because these indicators affect consumer preferences. The hot technique resulted in a stronger gummy jelly than the warm technique. This may be due to stronger intermolecular forces when the mixture was combined at high temperature. Adding 10-40% of honey weight of gelatin in all warm and hot (except for H4) techniques resulted in a decrease in hardness. A study showed that acid hydrolysis of gelatin contributed to the decrease in the strength of gelatin (Charoen et al., 2015). In this research, it may be due to the addition of more wheatgrass, which is reported to have an acidic pH of 5 (Rodriguez et al., 2022). The H4 formulation had the highest hardness possibly due to the gelatin content and high temperature applied, which led to higher level of gel cross-linking and network formation (Rebers et al., 2019). The chewiness and gumminess also showed the same trend as hardness. The results indicated that the concentration of 10% honey weight of gelatin using hot technique (H1) did not affect the hardness, gumminess and chewiness compared to 20-30% honey weight of gelatin using both hot and warm techniques. Based on the above information, H1 has suitable moisture, water, pH and color. Therefore, H1 is selected to study nutritional properties.

4. Nutritional properties

The nutritional properties of the H1 formulation were analyzed as shown in Table 4. Total energy of the H1 formulation was 56 kcal/50 g. Most of the energy of the gummy jelly was attributed to carbohydrate, which was 14 g and comprised of glucose (6 g) and fructose (8 g). The developed formulation showed a lower energy when compared to the commercially available formulations, reported by Teixeira-Lemos et al. at 175.5 kcal/50 g (Teixeira-Lemos et al., 2021). The world health organization (WHO) guideline recommended that adults and children should consume less than 25 grams of sugar per day (Huang et al., 2023). The guideline provides additional health benefits to reduce the risk of NCDs (WHO, 2015). Therefore, the gummy jelly was suitable for less than 2 servings/day.

Table 4 Nutritional properties of H1 gummy jelly

Nutritional properties	Nutritional values
Total energy, kcal/50 g	56
Carbohydrate, g (% of total energy)	14 (100)
Sugar, g	14
- Glucose, g	6
- Fructose, g	8
Protein, g (% of total energy)	0 (0)
Fat, g (% of total energy)	0 (0)

Conclusion

Gummy jellies were made from different gelatin concentrations using hot and warm techniques. The formulation used wheatgrass juice in the 50% effective concentration and replacing sugar with honey. Gummy jellies exhibited low water activity, moisture and pH, especially the H1 formulation. The reddish yellow of gummy jellies were probably derived from the pigments found in honey and wheatgrass that are beneficial to consumers. The hot technique resulted in a stronger gummy jelly than the warm technique. The gumminess was not different between the H1 formulation and all

Table 3 The texture profile of gummy jellies

Technique	properties					
	Hardness (g)	Adhesiveness^{ns} (mJ)	Cohesiveness^{ns}	Springiness^{ns} (mm)	Gumminess (g)	Chewiness (mJ)
W1	313.50±4.95^{b}	0.10±0.00	0.84±0.03	4.68±0.02	264.00±4.24^{b}	12.10±0.28^{b}
W2	288.00±1.41^{b}	0.10±0.14	0.87±0.06	4.71±0.0 2	248.50±16.26^{b}	11.50±0.71^{b}
W3	270.50±6.36^{b}	0.05±0.07	0.95±0.16	4.80±0.15	258.00±48.08^{b}	12.15±2.62^{b}
W4	233.50±7.78^{c}	0.40±0.57	0.82±0.32	4.66±0.15	181.50±44.55^{c}	8.30±2.26^{c}
H1	581.00±57.98^{b}	0.05±0.07	0.93±0.00	4.80±0.03	540.00±53.74^{b}	25.40±2.40^{b}
H2	449.00±0.00^{b}	0.01±0.00	0.90±0.01	4.77±0.11	405.00±5.66^{b}	18.95±0.78^{b}
H3	343.50±55.86^{b}	0.10±0.00	0.92±0.07	4.75±0.05	315.00±26.87^{b}	14.65±1.06^{b}
H4	1020.50±44.55^{a}	0.45±0.49	0.91±0.05	4.72±0.16	924.50±10.61^{a}	42.75±2.92^{a}

Remark: $^{a-c}$ different letters in the same column indicate values that are significantly different in statistics (p≤0.05), ^{ns} is not significantly different in statistics (p>0.05) in the same column.

other formulations, except for H4. The nutritional properties of the H1 formulation suggested that the gummy jelly was suitable for less than 2 servings/day. According to the above results, the H1 formulation (10% honey weight of gelatin with hot technique) could be recommended for a gummy jelly manufacturing process.

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