EJFA Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 36: 1–10
doi: 10.3897/ejfa.2024.125139

RESEARCH PAPER

Quality characteristics of chocolates incorporated with broccoli leaves jellies

Chi Hong Yeap[1], Mansoor Abdul Hamid[1,2], Oslida Martony[3], Titi Mutiara Kiranawati[4]

1 *Food Technology and Bioprocessing Program, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*

2 *Functional Foods Research Group, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS 88400, Kota Kinabalu, Sabah, Malaysia*

3 *Nutrition and Dietetic Program, Polytechnic of Healthy Medan, Jl. Jamin Ginting, Km 13.5, Kel. Lau Chi Medan, Tuntung 20136 Medan, Sumatera, Indonesia*

4 *Industrial Technology Department, Faculty of Engineering, Universitas Negeri Malang, Malang 65145 Indonesia*

Corresponding author: Mansoor Abdul Hamid (chot@ums.edu.my)

Academic editor: Maria Manuela Silva ♦ **Received** 10 April 2024 ♦ **Accepted** 16 August 2024 ♦ **Published** 26 November 2024

Abstract

Chocolate is a popular confectionery worldwide and thus, there is a need to improve the nutritional quality of chocolates. Broccoli leaves which are normally disposed as wastes were turned into the fillings of chocolates to enhance the quality of chocolates. This research aimed to propose the best formulation of chocolate incorporated with broccoli leaves jellies. Six formulations of broccoli leaves jellies (F1: 1.25%, F2: 5.00%, F3: 8.75%, F4: 12.50%, F5: 16.25% and F6: 20.00%) were incorporated into chocolates of fixed formulation. F2, with 5.00% of broccoli leaves was chosen as the best formulation on the basis of sensory evaluation and physiochemical tests which included water activity (a_w) and hardness tests. Subsequently, F2 was compared with the control from the aspects of proximate compositions, melting properties and antioxidant capacity. F2 recorded higher proximate compositions, except for moisture and crude fat content than the control. Higher melting properties, total phenolic and flavonoid contents together with a lower half maximal effective concentration (EC_{50}) were seen in F2 as compared to the control. Thus, the upcycling of broccoli leaves as food ingredients is a feasible one as proven in this study. Overall, we can also expect the valorization of broccoli leaves in the development of other functional food products.

Keywords

agricultural by-products, brassica, broccoli leaves, chocolate, innovative confectionery, jelly

Introduction

Consumers today are becoming more concerned about their health. Thus, the market today is introduced with many functional products and the current key trend is confectionery products that impart functional benefits to the consumers, for example functional chocolates and sugarless sweets (Hamid et al. 2021). Chocolate is defined as a semi-solid suspension of cocoa, sugar and milk powder (depending on the type of chocolate) in a continuous fat phase of cocoa butter, milk fat and emulsifiers (Afoakwa 2016). Chocolates have always been a hot topic of debate, as Tan et al. (2012) relate chocolates with great cardiometabolic health benefits whilst Veronese et al. (2019) argue the other way round. Consequently, researchers are always trying to improve the nutritional values of chocolates without deteriorating their organoleptic properties as chocolates, with unique flavour and taste are widely consumed worldwide as confectioneries, chocolate-coated products including cakes, biscuits, snacks, ice creams or beverages (Hamid et al. 2021). Approximately 7.3 million metric tons of chocolates are consumed each year (Gitnux 2023). To date, there are various functional chocolates in the market. For instance, chocolates incorporated with *Lactobacillus helveticus* and

Bifidobacterium longum known as Ohso probiotic chocolates, by the Belgian chocolate manufacturer, Ohso. There were also many other functional chocolates produced for research purposes, but yet to be commercialized, such as dark chocolate fused with flaxseed oil and honey (Singh et al. 2020), and dark chocolates incorporated with moringa oleifera leaf filling (Hamid et al. 2021) and so on. Hence, it can be said that developing functional chocolates is the current trend in the functional food market. Chocolates were therefore chosen as the matrix in this study due to its popularity and highly-acceptable organoleptic properties.

Broccoli (*Brassica oleracea* L. italica) belongs to the Brassicaceae family that includes cabbage, kale, Brussels sprout, savoy cabbage, gai lan, collard green and kohlrabi. A broccoli consists of three aerial parts, which are the floret (head), leaves and stem (include roots). Unfortunately, florets are the only parts that people usually consume because the consumer markets on the other parts of the plant can be considered as non-existent. It is said that only about 10–15% of the total aerial mass of broccoli is consumed, leaving the remaining parts of the plants as wastes (Liu et al. 2018). In fact, the upcycling of broccoli leaves should be practised in the food industry as the leaves have been repeatedly proven to be containing the highest contents of bioactive compounds and nutrients than the florets and stems (Liu et al. 2018; Devi et al. 2022). Although being categorized as the same family, broccoli leaves are considered underutilized in comparison to collard green despite having magnificent health benefits as mentioned (Liu et al. 2018). Necessary approaches are therefore required to valorize these underutilized broccoli portions instead of simply discarding them. They have high potential of being functional food ingredients applicable in food products and result in an improved nutritional values or functional properties of the food products. For example, gluten-free breads fortified with broccoli leaf powder showed an improvement in the technological properties and nutritional values of the product (Krupa-Kozak et al. 2021). This study was conducted to investigate the suitability of incorporation of broccoli leaves jellies into chocolates from the aspect of sensory properties and its effect on the physicochemical properties, proximate compositions, melting properties and antioxidant properties of the chocolates made therefrom.

Materials and methods

Providing materials

The materials used in preparing the broccoli leaves jellies, which included the agar-agar powder, citric acid, Beryl's dark and milk chocolate blocks, milk powder and castor sugar were purchased from a local bakery store known as Bake with Yen located at Lintas Square, Sabah.

Preparation of broccoli leaf powder

Broccoli leaves were obtained from Pasar Besar Kota Kinabalu, Malaysia and immediately sent to the laboratory in Faculty of Food Science and Nutrition, Universiti Malaysia Sabah for further processing. Broccoli leaves were washed thoroughly under running tap water followed by 10 minutes of blanching at 70 °C. Then, the leaves were cut into numerous small fractions and placed on trays covered with aluminum films and left for drying for 12 hours at 75 °C in a drying cabinet (TD-78T-2-D, Thermoline, Australia) which had been pre-heated for 30 minutes beforehand. The dried broccoli leaves were then ground into powder by using a blender (7010S, Waring, United States of America) and sieved using a mechanical sieve shaker (AS400, Retsch, Germany) with sieve having pore size of 125 µm. The powder was stored in a refrigerator (NR-BL342VSMY, Panasonic, Japan) at 4 °C until further use.

Preparation of broccoli leaves jellies

Water was heated to 70 °C for 5 minutes. Based on the formulations in Table 1, broccoli leaf powder, citric acid and sugar were added into it, stirred and boiled for 5 minutes. The mixture was added with agar-agar powder and stirred continuously followed by pouring into moulds and left to cool at room temperature for gel to set up. The jelly was dried in the cabinet dryer until 15 ± 0.05% of moisture content was obtained and cut into pieces of 1 ± 0.05 g. The jellies were refrigerated at 4 °C until further use.

Preparation of chocolates incorporated with broccoli leaves jellies

Table 1. Formulations of broccoli leaves jellies.

Formulations	Portion of ingredients (%)				
	Water	Sugar	Broccoli leaf powder	Agar-agar powder	Citric acid
F1	80.25	15.00	1.25	3.00	0.50
F2	76.50	15.00	5.00	3.00	0.50
F3	72.75	15.00	8.75	3.00	0.50
F4	69.00	15.00	12.50	3.00	0.50
F5	65.25	15.00	16.25	3.00	0.50
F6	61.50	15.00	20.00	3.00	0.50

The formulation of chocolate was depicted in Table 2. The chocolate was prepared by using double-boiling method where water was heated to 70 °C in a pot and a bowl was placed on top of it. The dark and milk chocolate compound blocks (Beryl's) were placed into the bowl and stirred until the chocolate blocks became molten. Then, milk powder and castor sugar were added and mixed by continuous stirring until a brown smooth paste was obtained. It was

Table 2. Formulation of chocolates.

Ingredients	Percentage (%)
Beryl's dark chocolate block	71.43
Beryl's milk chocolate block	17.86
Milk powder	7.14
Castor sugar	3.57

then transferred into a refiner (Stone Melanger 220V, Spectra) for refining process for 4 hours, followed by moulding where half the volume of mould was filled by the chocolate to act as the outer layer. The semimoist broccoli leaves jelly of 1 ± 0.05 g was added into the mould and covered by another half of molten chocolate. Aluminium foils were used to wrap the finished chocolates and chilled in a refrigerator at 4 °C until further use. Each chocolate without the filling was about 7 ± 0.05 g. Table 3 presents the overall compositions of all the formulations of chocolates.

Sensory evaluation

All the 7 formulations of chocolates were subjected to a sensory evaluation which involved a total of 53 untrained panelists that took place in a sensory evaluation room equipped with individual partitions to minimize any form of distractions. According to Stone and Sidek (2004), the recommended number of panelists in a laboratory testing should be between 25 to 50. Moreover, the panelists were those who have shown a keen sense of sensory perception towards chocolates in a prescreening session. On the day of sensory evaluation, the panelists were first explained with the products to be tested, the possible allergens in the products and the rules during the evaluation. Upon agreement, panelists were then instructed to be seated at each partition. They were served with 7 formulations of chocolates on a tray assigned with random 3-digit numbers and a cup of palate cleanser, in this case room-temperatured water. They were instructed to cleanse their palates before tasting and between each sample as well as to rate each sample immediately after tasting. 9-point hedonic scale which ranges from 1 (Dislike Extremely) to 9 (Like Extremely) was employed in this test to evaluate the attributes which colour, taste, aroma, after taste and overall acceptance.

Water activity (a_w) and hardness

The level of water activity of all the formulations was measured by a water activity meter (Hygrolabn C1, Rotronic, Switzerland) for three times to obtain an average value.

Textural analysis of chocolate samples was carried out as exhibited by Belščak-Cvitanović et al. (2012) with the aid of a texture analyzer (TA.XT plus, Stable Micro Systems, United Kingdom). The chocolate samples were left to cool down for 1 hour at room temperature prior to the analysis after being taken out from the refrigerator. 2 mm cylinder probe and a 10 kg load cell were used during the penetration at a speed of 1.0 mm/s and a distance of 7.5 mm. The hardness of the sample was indicated by gram (g) required to penetrate the sample. All the measurements were performed in triplicates.

Selection of best formulation

The best formulation was screened based on the sensory evaluation and physicochemical analyses that included water activity (a_w) and hardness test. Then, the best formulation was compared with the control from the aspects of proximal analyses, melting properties and total phenolic content, total flavonoid content and DPPH free radical scavenging activity (EC_{50}).

Proximate compositions

AOAC (2020) methods were used to determine the proximate compositions of the control and the best formulation chosen from sensory test and physicochemical tests. The proximate compositions analyzed were moisture content, ash content, crude protein, crude fat, crude fibre and carbohydrate. The content of carbohydrate was obtained by subtracting the sum of other values from 100%.

Melting properties

The melting properties of chocolates were examined based on that proposed by Oba et al. (2017). A differential scanning calorimeter (DSC) (DSC 8000, Perkin Elmer, United States of America) was used to determine the melting properties of the chocolate samples. Approximately 3 mg of sample was loaded into a 40 ml capacity pan and then sealed with a hermetic lid. The capacity pan was heated from 0 to 60 °C at the heating rate of 10 °C/min performed by nitrogen gas (N_2) stream. The onset temperature (T_{onset}), peak temperature (T_{peak}) and end temperature (T_{end}) were recorded. Melting index (T_{index}) was calculated by subtracting T_{end} from T_{onset} as proposed by Afoakwa (2016). The enthalpy, ΔH was obtained from the data shown.

Table 3. Overall compositions of all the formulations of chocolates.

Formulations	Chocolate (%)				Broccoli Leaves Jelly (%)				
	Dark chocolate	Milk chocolate	Milk powder	Castor sugar	Water	Sugar	Broccoli leaves powder	Agar-agar powder	Citric acid
F0	71.43	17.86	7.14	3.57	-	-	-	-	-
F1					80.25	15.00	1.25	3.00	0.50
F2					76.50	15.00	5.00	3.00	0.50
F3					72.75	15.00	8.75	3.00	0.50
F4					69.00	15.00	12.50	3.00	0.50
F5					65.25	15.00	16.25	3.00	0.50
F6					61.50	15.00	20.00	3.00	0.50

Total phenolic content, total flavonoid content and DPPH free radical scavenging activity

Preparation of chocolate sample extract was done as proposed by Belščak-Cvitanović et al. (2012). The chocolate samples were frozen and ground. 8.00 ± 0.01 g of each chocolate sample was extracted 3 times with 10 mL of n-hexane (Sigma-Aldrich, Malaysia) to remove lipids from the samples. The defatted chocolate samples were then air-dried for one day to remove any residual of organic solvent. 2.00 ± 0.01 g of each sample was extracted with 20 mL of 80% methanol (Merck Millipore, Malaysia) with the aid of ultrasound assisted extraction (Branson 8510, Branson, United States of America) for 30 minutes followed by centrifugation where the supernatant was decanted after the mixture was centrifuged at 6000 rpm for 10 minutes (MPR 16, Cole-Parmer, United States of America). The supernatants were stored in a freezer at -20 °C.

The analysis of total phenolic content was carried out as proposed by Pothitirat et al. (2009) with a slight modification. 0.2 mL of extract was mixed with 0.5 mL diluted Folin-Ciocalteu reagent (1:10 distilled water) (R&M Chemicals, Malaysia) and 0.5 mL of 7.5% (w/v) sodium carbonate solution (Sigma-Aldrich, Malaysia). The mixture was left at room temperature for 30 minutes with shaking in between. Using 765 nm of wavelength, the absorbance of mixture was measured by using a UV-vis spectrophotometer (Lambda 35, Perkin Elmer, United States of America). Gallic acid (Sigma-Aldrich, Malaysia) was used as the standard in this analysis and prepared by diluting the acid with methanol (Merck Millipore, Malaysia) to obtain the concentrations in the range of 0.10–0.80 mg/mL. The presence of total phenolic contents in each extract was expressed as mg gallic acid equivalents (GAE)/g of sample. All the measurement were repeated for three times.

$$T = cv / m \quad (1)$$

Where T is total phenolic content, c is concentration of gallic acid established from the curve, v is volume of extract solution and m is the mass of extract.

The quantification of total flavonoid content (TFC) of the samples was conducted as exemplified by Godočiková et al. (2016) with slight modifications. Specifically, an initial mixture of 0.5 ml of extract and 0.1 ml of 10% (w/v) ethanolic solution (Merk Millipore, Malaysia) of aluminium chloride (Sigma-Aldrich, Malaysia) was prepared, followed by the addition of 0.1 ml of 1 M sodium acetate (Sigma-Aldrich, Malaysia) and 4.3 ml of distilled water. After incubating the mixture for half an hour in darkness, the absorbance of mixture was taken through the use of a UV-vis spectrophotometer (Lambda 35, Perkin Elmer, United States of America) at 415 nm. The standard used in this test was quercetin (Sigma-Aldrich, Malaysia) with the concentrations in the range of 1–1.80 mg/mL. The results were indicated as mg quercetin equivalents (QE)/g of sample. The measurements were repeated thrice.

$$C = cv / m \quad (2)$$

Where C is the total flavonoid content, c is the concentration of quercetin established from the curve, v is volume of extract solution and m is the mass of extract.

Free radical scavenging method was done as described by Pothitirat et al. (2009) with a slight modification. DPPH solution was prepared by dissolving 0.1 mg of DPPH powder (Sigma-Aldrich, Malaysia) with 100 mL of 80% methanol (Merck Millipore, Malaysia) in a volumetric flask covered by aluminium foil and left for resting in a dark place at room temperature for 30 minutes. A series of extract concentrations were diluted (100–175 mg/ml). Then, measurements of the sample (2 mL of DPPH and 1 mL of extract) against the blank (2 mL of DPPH and 1 mL of methanol) were carried out using a UV-vis spectrophotometer (Lambda 35, Perkin Elmer, United States of America) at the wavelength of 517 nm. Their free radical scavenging activities were obtained and was subsequently used to build a graph of free radical scavenging activities against their corresponding concentrations. Half maximal effective concentration (EC_{50}) was determined based on the plotted graph.

$$\text{DPPHscavenging}\ (\%) = [(A_{control} - A_{sample)}/A_{control}] \times 100\% \quad (3)$$

Where $A_{control}$ is the absorbance of control reaction (2 ml of DPPH solution + 1 ml of methanol) and A_{sample} is the absorbance of test sample (2 ml of DPPH solution + 1 ml of extract).

Statistical analysis

All the collected data were analysed using the software Statistical Package for Social Sciences (SPSS) version 28 (IBM Corp). One-way analysis of variance (ANOVA) and post hoc Turkey test were used to analyse the data of 9-point hedonic scale, physicochemical analysis and storage data, whereas Independent Sample t-Test was used to analyse the data of proximate analysis, melting properties and antioxidant capacity. The significance level of SPSS was set at $p < 0.05$. Pearson's correlation coefficient (r^2) and p-value were used to show the correlations between total phenolic content (TPC), total flavonoid content (TFC) and DPPH free radical scavenging activity (EC_{50}) and their significance at $p < 0.05$.

Results and discussion

Sensory evaluation

Table 4 presents the sensory characteristics of all the formulations of chocolates. From the attribute of texture, F6 received the lowest mean score. This can be linked to the

Table 4. The sensory characteristics of all the formulations of chocolates.

Formulation	Attributes				
	Texture	Taste	Aroma	After taste	Overall acceptability
F0	7.77 ± 1.09^{a}	7.94 ± 1.13^{a}	7.81 ± 1.11^{a}	7.70 ± 1.27^{a}	7.83 ± 1.00^{a}
F1	7.61 ± 0.71^{a}	7.30 ± 1.31^{ab}	7.72 ± 0.74^{a}	7.06 ± 1.37^{abc}	7.47 ± 0.91^{ab}
F2	7.64 ± 0.79^{a}	7.79 ± 0.72^{ab}	7.83 ± 0.80^{a}	7.77 ± 0.67^{a}	7.85 ± 0.63^{a}
F3	6.47 ± 1.61^{b}	7.02 ± 1.54^{b}	7.70 ± 1.03^{a}	6.49 ± 1.78^{cde}	7.06 ± 1.11^{b}
F4	6.11 ± 1.88^{b}	7.17 ± 1.59^{ab}	7.66 ± 0.96^{a}	6.89 ± 1.74^{bcd}	7.00 ± 1.37^{b}
F5	5.79 ± 1.74^{b}	6.21 ± 1.16^{c}	7.68 ± 0.78^{a}	6.04 ± 1.86^{de}	6.33 ± 1.63^{c}
F6	5.77 ± 1.28^{b}	6.15 ± 1.45^{c}	7.94 ± 1.13^{a}	5.85 ± 1.51^{e}	5.89 ± 1.27^{c}

Values are expressed as mean (n = 3) ± standard deviation and values denoted by various superscripts in the same column differ significantly ($p < 0.05$). F0: Control, F1: 1.25% BLP, F2: 5.00% BLP, F3: 8.75% BLP, F4: 12.50% BLP, F5: 16.25% BLP, F6: 20.00% BLP. BLP: Broccoli leaves powder.

presence of stiff jelly inside the chocolates because of the undissolved insoluble dietary fibre during the preparation of jelly as broccoli leaves contained a significantly higher content of insoluble dietary fibre (23.8–30.6% of DW) than soluble dietary fibre (1.9–2.3% of DW) (Berndtsson et al. 2020). From the aspect of taste, the lowest mean score was seen in F6. Generally, the higher the concentration of broccoli leaves in the jelly, it was more likely to be associated with an undesirable vegetably bitter taste and pungent odour contributed by glucosinolates in the leaves (Higdon et al. 2007). Since most glucosinolates are only degraded at temperature above 110 °C as reported by Oerlemans et al. (2006), thus, there could be a high retention of glucosinolates after the leaves were cabinet-dried at 75 °C. At lower concentrations, such as F1 and F2, the bitter taste can still be masked by the sugar and citric acid used, giving a fusion taste of sourness and sweetness.

There was no significant difference ($p < 0.05$) among all the formulations in terms of aroma because the broccoli leaf powder was incorporated into the chocolates in the form of dried jelly, but not fusing together with the chocolate paste as one during processing. Thus, it had negligible effect on the aroma of the chocolate since the jelly was utterly covered by the hardened chocolate shell. F6 received the lowest mean score in the attribute of after taste. The after taste was possibly due to the intense vegetably bitter taste from glucosinolates since F6 contained the highest concentration of broccoli leaves and such taste might be unpalatable to the panelists because confectionery products are usually perceived as sweet. F2 obtained the highest overall acceptability score whereas the lowest mean score was seen in F6. There was no significant difference ($p < 0.05$) between F0, F1 and F2. This indicated that the incorporation of broccoli leaves jellies at these two concentrations (F1 and F2) was considered suitable and well accepted by the panelists from all the evaluated aspects.

Water activity (a_w) and hardness

Significant difference ($p < 0.05$) between control and all the other formulations from the aspect of water activity (a_w) was reported in Table 4. This is because the control sample was not incorporated with broccoli leaves jelly with the moisture content of 15 ± 0.05%. Nevertheless, all the other formulations were not prone to spoilage upon being produced as all showed water activity values lower than 0.6 that do not permit the growth of any microorganisms (Labuza et al. 2007).

The hardness of chocolate is a crucial property of chocolate as it is very much associated with the sensory quality of the chocolate (Hrivna et al. 2021). However, to date, there is no international consensus on the standard hardness of chocolates. F1, F2, and F3 recorded lower hardness values than the control sample. No significant difference ($p < 0.05$) was observed between F0 and F4. The difference in terms of the hardness was solely attributed to the presence of the broccoli leaves jelly as the formulation of the chocolate shell was fixed. Lower values of hardness as seen in F1, F2 and F3 can be attributed to the presence of soft broccoli leaves jellies. Since broccoli leaves contain higher proportion of insoluble dietary fibre than soluble dietary (Berndtsson et al. 2020), it is reasonable that the jellies became gradually stiffer at a higher concentration of broccoli leaves due to the accumulation of insoluble dietary fibre after being dried.

Selection of best formulation

F2 was chosen as the best formulation as it showed insignificant difference ($p > 0.05$) from F0 from the aspects of taste and texture and topped in three attributes in the sensory evaluation (aroma, after taste and overall acceptability). This proves that the addition of F2 broccoli leaves jelly resulted in enhanced organoleptic properties. Even though F2 recorded a significantly higher ($p < 0.05$) water activity (a_w) than F0. However, the value was still lower than 0.60, which did not allow the growth of any microorganisms (Labuza et al. 2007). F2 showed a softer texture than F0 but was considered acceptable as proven in the sensory data. Fig. 1 illustrates the chocolate incorporated with F2 broccoli leaves jelly.

Proximate composition

The proximate compositions of control and the selected formulation, F2 are revealed in Table 5. A significantly higher ($p < 0.05$) moisture content was seen in F2 than

Figure 1. Chocolate with formulation 2 jelly.

Table 5. Water activity and hardness of all the formulations of chocolates.

Formulations	Water activity (a_w)	Hardness (g)
F0	0.576 ± 0.003^a	3432.02 ± 7.41^c
F1	0.584 ± 0.002^b	2805.15 ± 70.66^e
F2	0.588 ± 0.002^b	2902.02 ± 26.94^{de}
F3	0.586 ± 0.005^b	3018.97 ± 36.61^d
F4	0.584 ± 0.002^b	3507.67 ± 93.48^c
F5	0.587 ± 0.002^b	3753.42 ± 29.24^b
F6	0.587 ± 0.002^b	4090.72 ± 91.20^a

Values are expressed as mean (n = 3) ± standard deviation and values denoted by various superscripts in the same column differ significantly ($p < 0.05$). F0: Control, F1: 1.25% BLP, F2: 5.00% BLP, F3: 8.75% BLP, F4: 12.50% BLP, F5: 16.25% BLP, F6: 20.00% BLP. BLP: Broccoli leaves powder.

F0 due to the incorporation of broccoli leaves jelly. The result was in line with that of a past study that showed the addition of broccoli leaf powder into pasta at both concentrations (2.5% and 5%) increased the moisture content of their corresponding pastas (Drabińska et al. 2022b). Higher moisture content and lower hardness values and vice versa were reported in a past study by Farzanmehr and Abbasi (2009), and the same phenomenon was observed in this study as F2 showed a lower hardness than F0. The increase in moisture content of F2 can also be attributed to the increased quantity of crude fibre in the chocolates as high-water binding ability is exhibited by fibre through enclosure, polar and hydrophobic interactions and hydrogen bonding (Chaplin 2003). The addition of plant materials into a foodstuff can increase the crude fibre content because vegetables are a source of crude fibre, which is part of the insoluble fibre found in the edible portion of the cell walls of plants. However, both F0 and F2 were categorised as low moisture foods as both had moisture contents lower than 25% (Erkmen and Bozoglu 2016).

The ash contents did not differ significantly ($p < 0.05$) between the two formulations. This scenario might be due to the loss of minerals during the preparation of jelly caused by washing and blanching as leaching of minerals would occur during washing, blanching and cooking (Nabrzyski 2017). F2 showed a significantly higher ($p < 0.05$) protein content than F0. This is because of the presence of broccoli leaves jelly in the chocolates as broccoli leaf powder is characterized as a rich source of protein, with approximately 29% of protein content (Drabińska et al. 2018), with albumin being the protein fraction with the highest weightage in broccoli leaves (Sedlar et al. 2020), which is crucial for the maintenance of appropriate osmotic pressure, neutralisation of free radicals and binding and carry of substances including hormones, drugs, fatty acids, ions, etc. (Miller and Jedrzejczak 2001).

A lower fat content was detected in F2 than F0 because a portion of the chocolate was substituted by the broccoli leaves jelly. According to Drabińska et al. (2022a), the fat content of broccoli leaves was only about 4%. Besides, Bhandari et al. (2013) stated that the highest proportion of polyunsaturated fatty acids (PUFAs) was observed in broccoli leaves in comparison to the other aerial parts. A higher carbohydrate content was seen in F2 than F0 even though there was no significant difference ($p < 0.05$) between these two formulations. Results obtained here matches study by Rada et al. (2020) which reported an increased carbohydrate content in chocolates fused with spices as compared to the control. The increase in carbohydrate content in this research can be largely attributed to the significantly higher content of crude fibre present in F2 as crude fibre is a type of non-digestible carbohydrate (Lunn and Buttriss 2007).

Table 6. Proximate composition of control and formulation 2 chocolates.

Proximate composition (%)	F0	F2
Moisture content	0.93 ± 0.61^b	3.87 ± 0.31^a
Ash	2.13 ± 0.05^a	2.37 ± 0.31^a
Crude protein	6.82 ± 0.10^b	7.83 ± 0.06^a
Crude fat	34.50 ± 0.50^a	23.50 ± 0.50^b
Crude fibre	1.42 ± 0.20^b	4.43 ± 0.08^a
Carbohydrate	54.20 ± 0.58^a	57.00 ± 1.94^a

Values are expressed as mean (n = 3) ± standard deviation and values denoted by various superscripts in the same row differ significantly ($p < 0.05$). F0: Control, F2: 5.00%. BLP: Broccoli leaves powder.

Melting properties

Increment in all the melting properties from F0 to F2, which had a lower fat content was observed. Such data were in agreement with a past study that reported an increase in all the melting attributes from 50% fat-containing chocolates to 10% fat-containing chocolates (Tan and Kerr 2017). Low-fat chocolates (<25%) takes a longer time to melt and melt completely at a higher temperature than high-fat chocolates (30–35%) due to reduced free-flowing plastic flow and particle interaction in chocolates of a lower fat content (Afoakwa 2016). When fat content decreases, the distance between the solid particles decreases and this leads to the increase in the viscosity of chocolates due to higher inter-particle interactions in the chocolates (Servais et al. 2002).

Apart from that, it is also influenced by the moisture content. The flowability of a chocolate was influenced when a chocolate has a moisture content of more than 2%. F2 showed a moisture content of more than 2%. Such moisture content will cause sticking of sugar particles, impeding the flowability of chocolates and leads to an increase in the viscosity of chocolates (Kusumadevi et al. 2021). The positive values of ΔH indicates that the whole system was endothermic in nature because external supply of heat was absorbed by the samples to cause melting of chocolates. On top of that, since both formulations showed onset temperatures between 25.30 ± 0.02 and 25.57 ± 0.24 °C, this implies that the crystals formed in the chocolates should be form III. The assumption of crystals formed in this study is based on past reviews by Ghazani et al. (2021) and Talbot (2009) which stated that the melting temperatures of form III crystals are 25.5 °C. Overall, the melting properties of both F0 and F2 were considered normal as Afoakwa et al. (2008) mentioned that the expected melting profiles of chocolates should fall between 15 to 55 °C. This indicates that the incorporation of broccoli leaves jelly did not deteriorate the melting properties of chocolates, yet enhanced its heat resistance as indicated in Table 7.

Table 7. Melting properties of control and formulation 2 chocolates.

Melting Properties	Formulations	
	F0	F2
T_{onset} (°C)	25.30 ± 0.02^a	25.57 ± 0.24^a
T_{peak} (°C)	29.65 ± 0.23^a	30.61 ± 0.39^a
T_{end} (°C)	31.74 ± 0.42^b	37.32 ± 0.49^a
T_{index} (°C)	6.43 ± 0.44^b	11.75 ± 0.73^a
Entahlpy (ΔH) (J/g)	19.74 ± 0.08^a	20.37 ± 0.42^a

Values are expressed as mean (n = 3) ± standard deviation and values denoted by various superscripts in the same row differ significantly ($p < 0.05$). F0: Control, F2: 5.00%. BLP: Broccoli leaves powder.

Total phenolic content, total flavonoid content and DPPH free radical scavenging activity

Table 8 conveys the total phenolic content (TPC), total flavonoid content (TFC) and DPPH free radical scavenging activities (EC_{50}) of both control and F2. In a past research manifested by Krupa-Kozak et al. (2021), gluten-free bread fortified with 5% of broccoli leaf powder exhibited a higher TPC than its corresponding control. Similar results were revealed here where the increase in TPC observed in F2 was almost 2.3-fold higher than F0. The surge in the TPC content can be credited to the incorporation of broccoli leaves jelly because broccoli leaves are rich in phenolic compounds, and in fact is in an amount higher than that of florets and stalks as demonstrated by Liu et al. (2018). Interestingly, the TPC of F2 is lower than the average TPC of commercial dark chocolates in the Turkish market (Övet 2015). This can be attributed to the addition of milk powder in this study. According to Tabernero et al. (2006), presence of milk may influence the analysis of polyphenols as polyphenols can bind to milk proteins, forming protein–polyphenol complexes that can reduce the biological accessibility of the polyphenols, and subsequently the antioxidant capacity of chocolates. Flavonoids and hydroxycinnamic are the most widespread polyphenols present in Brassica species (Soengas et al. 2012). Polyphenols are crucial for human health as multidirectional antioxidant activities are shown by them, including removing free radicals and reactive oxygen species, preventing the formation of reactive oxygen species by inhibiting the activity of related enzyme, preventing enzymatic and non-enzymatic lipid peroxidation and acting as complexing agents for iron and copper, thereby preventing oxidation of ascorbic acid (Scalbert et al. 2005).

Flavonoids are a representative class of plant pigment present in fruits and vegetables by nature, which are unable to be produced by human bodies and act as antioxidants on biological systems (Calado et al. 2015). They are also a dominant class of phenolic compounds linked to a remarkable bioactivity on chronic venous insufficiency (Pinto et al. 2021). As reported in Table 7, the addition of broccoli leaves jelly into the chocolates successfully improved the total flavonoid content (TFC) of chocolates significantly ($p < 0.05$) by almost 2.6-fold as seen in F2 in comparison to F0. Broccoli leaves can be regarded as a valuable source of flavonoid because the leaves contained the highest flavonoid content than the stems and florets as outlined in a past investigation (Devi et al. 2021). Similar results were seen in a past study where dark chocolates incorporated with mulberry and sea buckthorn showed greater TFC than the control (Godočiková et al. 2017). However, the TFC of F2 is lower than the TFC of commercial dark chocolates in the Turkish market, which was 5.681 ± 0.49 mg QE/g (Övet 2015). This can be the result of the addition of milk powder in making the chocolates as mentioned earlier. Nevertheless, in general, the TFC of F2 is still higher than the average TFC of different types of commercial dark, milk and white chocolates available in the Malaysian market (Meng et al. 2009), milk chocolates in the Turkish market (Övet 2015) and milk chocolates sold in Peru (Calixto-Cotos et al. 2018), suggesting that F2 can be a healthier substitute to the consumers since flavonoids can act as a therapeutic agent by possessing neuroprotective, cardioprotective, antioxidant, anticancer, antiviral and anti-inflammatory properties (Ullah et al. 2020).

F2 showed a significantly lower concentration ($p < 0.05$) to achieve half maximal effective concentration (EC_{50}) than F0. The scenario can be attributed to the presence of broccoli leaves jelly that contributed to the higher content of phenolic compounds. There is a high correlation between total phenolic content (TPC) and antioxidant activity measured by DPPH assay as mentioned by Soengas et al. (2012). The same trend is observed in this study as expressed in Table 9, where high negative correlations between total phenolic content (r^2=-0.999), total flavonoid content (r^2=-0.981) and DPPH free radical scavenging activity (EC_{50}) were found, implying that these bioactive compounds and antioxidant capacity have a statistically significant linear relationship. In other words, the antioxidant capacity of the chocolates is heavily dependent on the bioactive compounds present in the chocolates.

The reduction of EC_{50} in F2 was nearly 17%. Since polyphenols can remove free radicals Scalbert et al. (2005), a higher content of TPC means more polyphenols are available to scavenge commercially available DPPH free radicals, resulting in a lower concentration needed to change the solution from violet to yellow. Apart from that, Liu et al. (2018) reported that highest DPPH radical scavenging activity was found in broccoli leaf tissues (30.7%), followed by stem tissues (16.4%) and finally floret tissues (14.7%). On top of that, carotenoids, especially β-carotene and lutein which are categorized as strong antioxidants are present in broccoli leaves alongside vitamins E (α- and γ-tocopherols) and K which have strong antioxidant properties. These statements further prove that broccoli leaves are considerably high in antioxidative property.

Table 8. Total phenolic and flavonoid contents and DPPH free radical scavenging activity (EC_{50}) of control and formulation 2 chocolates.

Property	F0	F2
TPC (mg GAE/g)	2.09 ± 0.06^{b}	4.78 ± 0.20^{a}
TFC (mg QE/g)	0.80 ± 0.08^{b}	2.07 ± 0.09^{a}
DPPH (mg/ml)	152.19 ± 0.50^{a}	126.37 ± 1.27^{b}

Values are expressed as mean (n = 3) ± standard deviation and values denoted by various superscripts in the same row differ significantly ($p < 0.05$). F0: Control, F2: 5.00%. BLP: Broccoli leaves powder.

Table 9. Pearson correlation (r^2) between total phenolic, flavonoid contents and DPPH free radical scavenging activity (EC_{50}).

	Fraction	Pearson correlation (r^2)	P-value
DPPH (EC_{50})	TPC	-0.999	<0.001
	TFC	-0.981	0.001

Conclusion

The current study disclosed the feasibility of adding broccoli leaves jellies into chocolates as a filling. Among all the six proposed formulations, formulation 2 (F2) was elected as the best formulation based on sensory evaluation and two physicochemical analyses, namely water activity (a_w) and hardness tests. In comparison with the control (F0), F2 showed higher proximate compositions, except for crude fat which implied that the fat content of the chocolates was markedly reduced. Besides, greater melting properties were observed in F2 than F0, suggesting an improvement in the heat resistance of the chocolates. Moreover, significantly higher bioactive compounds, namely total phenolic and flavonoid contents as well as free radical scavenging activity were observed in F2 than F0. This reveals that the incorporation of broccoli leaves jellies into chocolates ameliorated the chocolates' ability to scavenge free radicals, which has potential implications for promoting health and preventing chronic diseases. The research's findings support the notion that the addition of jellies made from 5.00% of broccoli leaves into chocolates offers a promising approach in improving the nutritional profile and antioxidant capacity of chocolates without sacrificing the organoleptic qualities of chocolates. Consequently, this could serve as an alternative to the commercial chocolates in the market for those health-conscious consumers. Apart from that, utilization of broccoli leaves can also contribute to the food security issue and help in reducing environmental issues as a result of simply disposal of side streams of vegetables. Further research should be conducted to further exploit the functional or technological properties of broccoli leaves in the related fields.

Author contributions

Concept and design: all authors. Analysis and interpretation: Chi Hong Yeap, Mansoor Abdul Hamid, Oslida Martony. Data collection: Chi Hong Yeap . Writing the article: Chi Hong Yeap. Critical revision of the article: All authors. Final approval of the article: All authors. Statistical analysis: Chi Hong Yeap, Mansoor Abdul Hamid, Titi Mutiara Kiranawati. Overall responsibility: Mansoor Abdul Hamid.

Conflict of interest

The authors declare no conflict of interest in this research.

Acknowledgements

The authors would like to express their greatest gratitude to Mr. Awang and Ms. Ina from the Kota Kinabalu Big Market for their generous and continuous supply of broccoli leaves used in this study.

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