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Characteristics of yogurt supplemented with different concentrations of *Carissa carandas* L.

Ni-orn Chomsri[1]* and Kamonwan Manowan[2]

[1] Agricultural Technology Research Institute, Rajamangala University of Technology Lanna
[2] Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna

***Corresponding author**: *niornchomsri@rmutl.ac.th*
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Abstract

Yogurt is a fermented dairy product claimed to confer health benefits due to the remaining viability of good lactic acid bacteria (LAB). This study investigates the influence of using *Carissa carandas* L. (CC) as a supplement in the yogurt. The yogurt samples were examined for their physicochemical, microbiological and sensory properties. Supplementation of CC berry in the yogurt improved the quality compared with 0% supplementation of CC berry. Yogurt with 20% CC berry exhibited the highest total phenolic compounds. LAB present in yogurt was remained above 8 log CFU/g after 21 day storage period at 4 ºC. Yogurt with CC berry supplements showed better sensory properties (moderately like to like very much) than yogurt without CC berry supplement ($p \leq 0.05$). The finding of this study suggests that CC berry is a potential plant-based component to improve both phytochemical and sensory properties in yogurt production.

Keywords: *Carissa carandas*, yogurt, lactic acid bacteria, phytochemicals

Introduction

Carissa carandas L. (CC) is an evergreen and spiny shrub belonging to Apocynaceae family (Weerawatanakorn and Pan, 2016), widely known in many local names, e.g. Carandas (Bansal, 2014), Karanda and Carunda (Kubola et al., 2011), Karaunda (Mehmood et al., 2014) Karamcha (Khatun et al., 2017). In Thailand, it is called Namdaeng (red thorn) or Manao Mai Ru Ho (Yuenyongphutthakal et al., 2012; Pewlong et al., 2014; Chomsri et al., 2018). The fruit turns from pinkish white to blackish purple when ripe and can be eaten raw or processed (Chomsri et al., 2018; Chomsri and Manowan, 2019). Mature fruit is useful for making jellies, jams, squash, syrup and chutney due to its high pectin content (Lapsongphon and Changso, 2019). The blackish purple fruit contains high composition of anthocyanin which is identified as cyanidin 3-rhamnoglucoside (Mohammad and Ding, 2019). The interest in CC has increased during the last decade especially studies on health effects because it is well recognized as an effective source of phytochemicals with excellent health benefits (Chomsri et al., 2017). Bhadane and Patil (2017) reported that the ripened CC berry was rich in flavonoids, e.g. rutin, epicatechin, quercetin and kaempferol including phenolics, e.g. syringic acid, vanillic acid and caffeic acid. Research on drying of CC berry pomace revealed its alternative use of the tablet product (Yuenyongphutthakal et al., 2012). Many reports claim pharmacological characteristics of CC such as treatment of constipation and diarrhea, stomachic, anorexia, intermittent fever, mouth ulcer and sore throat, syphilitic pain, burning sensation, scabies and epilepsy (Mehmood et al., 2014, Khatun et al., 2017; Bahdane and Pati, 2017).

Yogurt is a fermented dairy product, produced by the activity at 1:1 ratio of two well-known species of LAB, i.e. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* converting lactose in milk to lactic acid and other flavor compounds under a controlled temperature and environmental conditions (Das et al., 2019). This results in a unique characteristics present in the finish product. The drop in pH caused by the production of lactic acid affects milk proteins to denature, providing yogurt with its characteristic texture and sour flavor (Hekmat and Reid, 2006). Nowadays, a strong focus on healthful, natural, and nutritional offerings on food influences millennial consumer behavior resulting in new development in food industry, including yogurt production. Various plant based components are combined into yogurt for consumer choice, which is generally associated with a healthier product (Sah et al., 2016; Barkallah et al., 2017; Tavakoli et al., 2018; Silva et al., 2019; Wang et al., 2020). In addition, sensory aspect has been considered very important to enhance consumption (Perina, 2015; Abdel-Hamid et al., 2020).

CC can exert several pharmacological effects because of their content of bioactive phytochemical compounds (Bhadane and Patil et al., 2017; Chomsri et al., 2017). Hence the aim of this

https://doi.org/10.14456/jsat.2020.3

study was to evaluate the performance of CC supplement in yogurt production. The influence of CC contents on physicochemical, microbiological and sensorial characteristics of produced yogurt was investigated.

Materials and methods

CC berry preparation

Fully ripe with blackish purple and sound CC was collected from Agricultural Technology Research Institute, Rajamangala University of Technology Lanna, Lampang, Thailand. After washing, sorting and deseeding, the CC flesh was mixed with sucrose at the ratio of 1:1 and heated at 90-95 ^{o}C for 20 minutes.

Yogurt preparation

Yogurt samples were prepared by heating cow's milk at 90-95 ^{o}C for 10 min and cooled down by 45 ^{o}C. The milk was inoculated with commercial yogurt culture (Lyofast, Sacco Srl, Italy). The mixture of milk and starter yogurt culture was incubated at 45 ^{o}C for 5 h or pH value was less than 4.5. Subsequently, CC berry contents at 4 levels based on yogurt (w/w), i.e. 0% (YCC0), 10% (YCC10), 15% (YCC15), and 20% (YCC20) were added and mixed with the plain yogurt. The CC yogurt samples were then packed in cups of 30 ml and stored at 4$\pm$1 °C before analysis at 0, 7, 14 and 21 days.

Microbiological enumeration

For the enumeration of viable cell counts, 10 g samples were collected directly after packing and storage for 24 h. The samples were serially diluted in 90 mL sterile Ringer's solution (NaCl 1.125 g/l, KCl 0.0525 g/l, $CaCl_2$ 0.03 g/l and $NaHCO_3$ 0.025 g/l). The diluted samples were plated on MRS (Merck, Darmstadt, Germany) and PCA (Merck, Darmstadt, Germany) for LAB and total bacterial count, respectively.

Chemical Analysis

pH was measured by digital pH meter (Model C831, Belgium) according to Nielsen (2017). Total acidity was determined by diluting each 5 ml aliquot of sample in 50 ml distilled water and then titrating to pH 8.2 using 0.1 N NaOH (Iland et al., 2000). Titratable acidity was expressed as citric acid and lactic acid percentages for CC berry and yogurt, respectively. Total soluble solid content was determined on an Atago hand-held refractometer. Free alpha amino nitrogen (FAN) was quantified by spectrophotometric method (Intaramoree and Chomsri, 2014) using the spectrophotometer (T80 UV, PG Instrments, England). Total anthocyanin content was evaluated by the pH-differential method of Giusti and Wrolstad (2005). The Folin-Ciocalte method was used to evaluate total phenolic content (Spínola et al., 2015). The antioxidant activity was determined by the ABTS method (Wongputtisin et al., 2007). The colorimetric protein assay was used to measure the concentration of total soluble protein (Bradford, 1976). Total sugar content was measured by phenol-sulfuric acid method (Nielsen, 2017).

Syneresis and water-holding capacity (WHC) evaluation

Syneresis defined as the formation of a liquid (whey) on top of the yogurt was measured using the centrifugation method according to Farnworth et al. (2006) with modification. 40 ml of yogurt was centrifuged at 500 x g for 10 min at 4 ^{o}C. The clear supernatant was collected and weighed. The syneresis percentage was calculated as the percentage of separated whey from gel network during centrifuge. Water holding capacity (WHC) was measured as described by modified method of Serra et al. (2007) and Michael et al. (2010). 40 ml of yogurt was weighed and centrifuged at 5,000 *x g* at 10 ^{o}C for 20 min. After centrifugation, separated supernatant was drained, whey was weighed using a sieve in order to ensure the absence of any curd particles. WHC was expressed as the percentage of grams of expelled whey per gram of yogurt. These measurements were performed in triplicate.

Sensory analysis

All the panelists were experienced in yogurt. A group of 30 panelists took part in this study. Yogurt products fermented for 5 h and stored for 24 h were evaluated for organoleptic quality. The samples of yogurt were served in random order at 4 °C in plastic cups identified with a random 3-digit code. The panelists were suggested to rinse their mouths with water between samples. Each panelist received 4 samples of yogurt to evaluate and comment on the sensory characteristics. The panelists were asked to evaluate the appearance, color, odor, flavor, texture and overall preference of the final product depending on a nine-point hedonic scale, ranging from 9 (like extremely liked) to 1 (extremely disliked) for each organoleptic characteristic (Meilgaard et al., 2006).

Statistical analysis

All the experiments were carried out with 3 replications. Analysis of variance (ANOVA) was used to compare mean differences of the samples. Significant differences between treatments were analyzed by Duncan's new multiple range test (DNMRT) at a 0.95 significance level. Values were expressed as the mean of all replicate determinations with standard deviation.

https://doi.org/10.14456/jsat.2020.3

Results and discussion

Composition of CC berry

The results showed that the pH and acidity of CC fruit were 3.11 and 1.71%, respectively, therefore, it could be classified in the group of high acid food (Potter, 1986). According to the total soluble solid (TSS) content of 10 ºBrix, total sugar content of 72.79 mg/g and reducing sugar content of 63.36 mg/g containing in the CC berry, the low ratio of TSS/sugar and acid was obtained. This means that the CC fruit possessed the tangy and sour taste. Total phenolic content of 707.27 mg/kg and anthocyanin content of 112 mg/kg were found in the CC fruit in this study while Chomsri et al. (2017) reported higher contents of these phytochemical contents. This could probably be due to the cultivar and the ripening stage. Regarding to CC properties, addition of the fruit into yogurt could possibly serve as a source for functional foods.

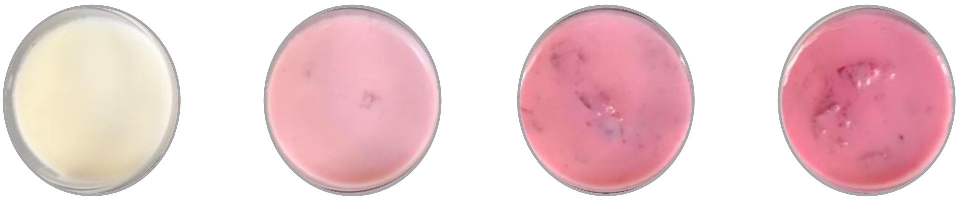
Table 1. Properties of *Carissa carandas* L. fruit

Properties	Values
pH	3.11±1.23
Total acidity (% as citric acid)	1.71±0.04
Total soluble solids (ºBrix)	10±2.58
Free α-amino nitrogen (mg/kg)	228±11.48
Total phenolic content (mg/kg)	707±29
Total soluble protein content (mg/kg)	82.17±2.65
Antioxidant activity (% ABTS scavenging effect)†	35.36±1.67
Total sugar content (mg/g)	72.97±5.84
Reducing sugar content (mg/g)	63.36±10.52
Total anthocyanin content (mg/kg)	112±6

† Data obtained from 100-fold dilution of the sample

Physicochemical properties of yogurt

Different colors of the yogurt from four treatments were obviously seen (Figure 1). Intensity of pinkish red color was increased in yogurt samples supplemented with higher CC berry contents. The pinkish red color was associated with concentrations of red pigments of anthocyanins, e.g. cyanidin-3-O-glucoside, cyanidin-3-O-galactoside and delphinidin-3-O-galacto-side containing in CC fruit as described by Mohammad and Ding (2019). As observed in this study, the anthocyanin components were likely to solubilize and stable in the yogurt matrix which seemed to improve the attribute of color appearance in the yogurt.



a b c d

Figure 1. Yogurt supplemented with different CC berry contents; a) 0%, b) 10%, c) 15% and d) 20%

As shown in Table 2, the effect of CC supplements on pH values, titratable acidity and total solid contents were observed. Four yogurt treatments in this study reached essential quality of minimum titratable acidity of 0.6% expressed as lactic acid (Codex Alimentarius Commission, 2011). The yogurt product after fermentation had pH in the range of 3.43-3.91. The yogurt sample without CC berry supplement showed the highest pH value of 3.91 which was similar to previous reports of Bosnea et al. (2017) and Delgado-Fernandez et al. (2019). In contrast, yogurt samples with CC berry supplement had lower pH values. This reduction was likely due to acids containing in CC berry. Yogurt containing higher contents of CC berry displayed significantly higher titratable acidity and total soluble solids ($p \leq 0.05$). This probably caused by the added sugar during CC preparation and solid parts found in CC fruit such as organic acids, sugars, pigments and fibers (Sarkar et al., 2018; Mohammad and Ding, 2019). Acids found in the yogurt samples were possibly derived from two sources, i.e. fermentation

https://doi.org/10.14456/jsat.2020.3

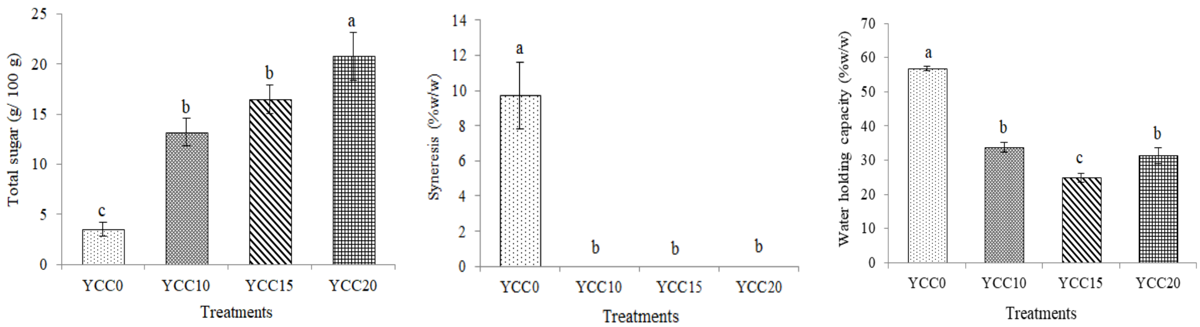
of lactose to organic acids such as lactic acid and acetic acid (Delgado-Fernandez et al., 2019) and organic acids existing in CC berry as described above. The concentration of FAN in the yogurt samples of this study were between 68.35-72.49 mg/kg while milk was reported to have FAN in the amount of approximately 0.50 mg/kg based on the average molecular weight of an amino acid (Farvin et al., 2010; McPherson and Pincus, 2011). This could indicate the formation of free amino acids and peptides resulting from bacterial proteolysis during fermentation which is in accordance with previous findings (Serra et al., 2009; Farvin et al., 2010). Total soluble protein contents were dramatically decreased in yogurt supplemented with CC berry which was possibly caused by reduction of plain yogurt content and protein–phenolic interactions. (Rodtjer et al., 2019; Ozdal et al., 2013; Isik et al., 2014; Seczyk et al., 2019). This binding was stronger at low pH condition in yogurt leading to change in characteristics of protein and phenolic compounds as well as its functional properties.

Table 2. Chemical property of yogurt supplemented with different CC berry contents

Treatment	pH*	TA (%)*	TSS (°Brix)*	FAN (mg/kg)ns	TSP (mg/kg)*
YCC0	3.91±0.04a	0.72±0.02b	6.00±0.00c	72.49±0.71	3810±417a
YCC10	3.71±0.02b	0.80±0.04b	17.00±0.00b	69.26±2.71	200±10b
YCC15	3.59±0.08b	1.00±0.07a	21.75±3.18b	68.66±1.00	189±17b
YCC20	3.43±0.02c	1.07±0.02a	27.00±1.41a	68.35±1.43	165±24b

ns denotes means are not significantly different ($p>0.05$)
* Means in a column with the different letters represent significant differences ($p\leq0.05$)
Titratable acidity (% as lactic acid); TA, total soluble solids (°Brix); TSS, free alpha amino nitrogen (mg/kg); FAN, total soluble protein (mg/kg); TSP. Yogurt samples containing CC berry contents of 0% (YCC0), 10% (YCC10), 15% (YCC15) and 20% (YCC20).

Figure 2a shows the total sugar contents, syneresis and WHC in yogurt samples. Adding CC berry content of 20% led to the highest total sugar content compared with that of lower CC berry contents. It was obvious that sugar containing in the prepared CC berries was the main reason of the sugar increase. Syneresis, the whey separation from milk protein gel is considered as an important index of yogurt quality (Vareltzis et al., 2016). Yogurt sample without CC berry supplement was high susceptibility while syneresis amount was not observed in the samples with CC supplements (Figure 2b). Creation of a binding water through solids containing in CC supplements could be a reason of the syneresis improvement. A similar observation was reported by Akgün et al. (2020). In contrast, the yogurt with higher concentrations of carrot juice resulted in higher syneresis susceptibility (Kiros et al., 2016). It is worth noting that syneresis of CC yogurt in this experiment was lower 4 to 6 times compared to other plant based yogurt (Kiros et al., 2016; Akgün et al., 2020). Figure 2c illustrates WHC of the CC yogurt samples. It appeared that CC supplement likely lowered WHC in yogurt samples. WHC of CC yogurt in this study were in agreement with other plant based yogurt (Barkallah et al., 2017; Mousavi et al., 2019). Vareltzis et al. (2016) revealed that increasing total solids reduced pore size in the protein matrix of the yogurt gel which led to a reduction in syneresis and improvement of the water-holding capacity of the gel.



a b c

Figure 2. Total sugar contents (a), syneresis (b) and water holding capacity (c) of yogurt supplemented with different CC berry contents; YCC0, 0%; YCC10, 10%; YCC15, 15% and YCC20, 20%.

https://doi.org/10.14456/jsat.2020.3

Table 3 indicates the significant difference of phytochemical values in the yogurt samples. Yogurt supplemented with 20 % CC berry gave the best phytochemical profiles ($p \leq 0.05$) which was correlated with anthocyanin pigments and phenolic compounds containing in CC fruit (Pewlong et al., 2014; Weerawatanakorn and Pan, 2016; Chomsri et al., 2017; Sarkar et al., 2018). These substances were most typically responsible for the antioxidant activity exhibited in the CC yogurts. Previous reports showed variability of phytochemical values in yogurt which could possibly explained by many factors, e.g. types of plant, supplement amount, preparation during processing, analysis method. For examples, yogurt supplement with peppermint, carrot and green tea showed total phenolic compounds of 30 (Amirdivani and Baba, 2011), 36 (Kiros et al., 2016) and 3220 (Muniandy et al., 2016) mg GAE/ kg, respectively. It is interesting to note that yogurt samples with CC supplements produced remarkable antioxidant activity and total phenolic contents compared to other plants based yogurt (Kiros et al., 2016; Akgün et al., 2020; Pan et al., 2019).

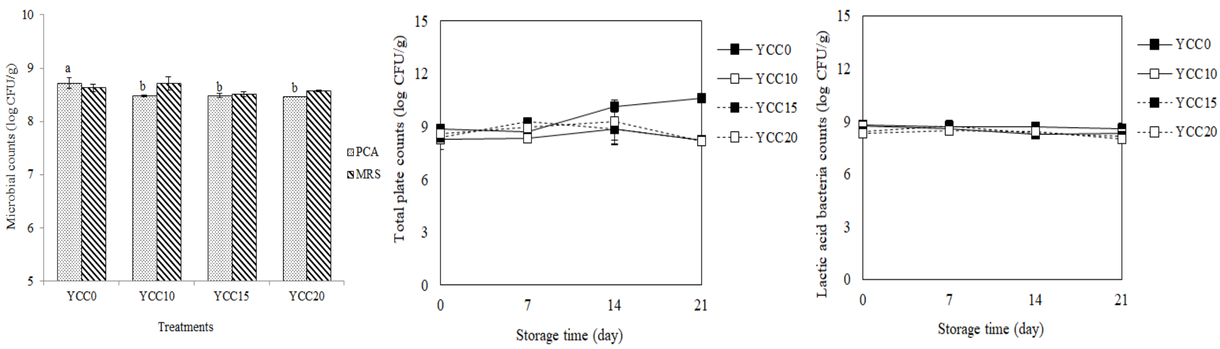
Table 3. Phytochemical contents and antioxidant activity of CC berry yogurts

Treatment	AO (%)*	AOA (mg/100g)*	AOT (mg/100g)*	TPC (mg GAE/kg)*
YCC0	23.62±0.43[d]	6.43±0.12[d]	9.10±0.16[d]	106±12[c]
YCC10	61.86±2.39[c]	16.86±0.65[c]	23.84±0.92[c]	306±7[b]
YCC15	73.30±1.26[b]	19.98±0.34[b]	28.26±0.49[b]	323±1[b]
YCC20	95.29±0.98[a]	25.68±0.26[a]	36.73±0.37[a]	446±45[a]

* Means in a column with the different letters represent significant differences ($p \leq 0.05$)
Antioxidant activity (% scavenging effect); AO, ascorbic acid equivalent antioxidant capacity (mg/100g); AOA, trolox equivalent antioxidant capacity (mg/100g); AOT, total phenolic content (mg GAE/kg); TPC, yogurt supplemented with different CC berry contents; YCC0, 0%; YCC10, 10%; YCC15, 15% and YCC20, 20%.

Microbiological analysis

Total viable count and LAB count in yogurt samples are presented on Figure 3. Initial bacterial counts indicated LAB present in yogurt samples between 8.5-8.7 log CFU/g which typically meets recommendation of at least 6 log CFU/g at time of consumption (Codex Alimentarius Commission, 2011; Das et al., 2019). Furthermore, it was revealed that LAB survivability in yogurt samples stored at 4 ºC for 21 days was obviously remained stable. This finding supports existing of living LAB in CC yogurt which could probably provide health benefit regarding its potential properties of specific strains, e.g. probiotic property, producing bioactive metabolites (Das et al., 2019).



a b c

Figure 3. Initial total viable count and LAB count (a) in CC yogurt, changes of viable counts (b) and LAB counts (c) in yogurt supplemented with different CC berry contents; YCC0, 0%; YCC10, 10%; YCC15, 15% and YCC20, 20%.

Sensory analysis of yogurt

Sensory properties of yogurt, particularly flavor and texture/ mouthfeel are important for consumer acceptance (Das et al., 2019). In this study, addition of the prepared CC had significant effect of on the attributes of the sensory characteristic in yogurts. Yogurt with CC berry supplements displayed better sensory qualities ($p \leq 0.05$). Mean hedonic scores of odor, flavor and overall preference attributes were significantly higher in the yogurts containing CC berry reflecting the improved organoleptic properties, resulting from CC berry supplementation to the experimental yogurt. This might be explained by positive combination of CC berry and sugar containing in the prepared CC berry

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could enhance greater acceptability in the yogurt product. Based on sensory attributes presented in this study, the yogurt with 15% CC exhibited the best acceptance.

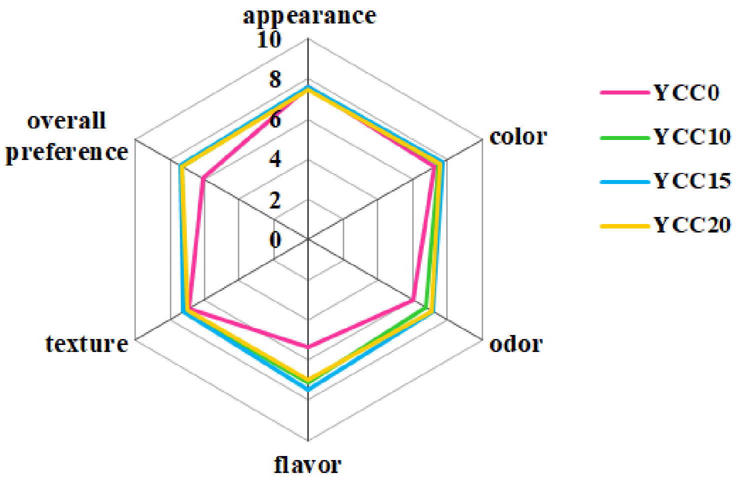

Figure 4. Effect of CC berry supplementation on sensory quality of yogurt supplemented with different CC berry contents; YCC0, 0%; YCC10, 10%; YCC15, 15% and YCC20, 20%.

Conclusion

In conclusion, CC berry supplementation in yogurt gave positive effects to yogurt product quality. Yogurt with 20% CC berry exhibited the highest phytochemical properties of phenolic compounds and antioxidant activity whereas yogurt with 15% CC berry showed the highest sensory properties. In addition, LAB survival throughout the storage period is a promising evidence to warrant further development of CC based product to obtain healthier yogurt.

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