

Development of Beta (β) Carotene Enriched Drinking Yoghurt by Incorporating Carrot (*Daucus carota*) Pulp and Orange (*Citrus sinensis*) Juice

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Abstract

Beta (β) carotene is a natural antioxidant which is omnipresent in yellow, green vegetables and fruits. The main objective of this study was to develop a β carotene enriched drinking yoghurt by incorporating carrot pulp and orange juice. The mixing ratio of carrot pulp, orange juice and yoghurt base was selected by analyzing the data gathered from a preliminary sensory trial with respect to appearance, colour, taste, mouth feel, and overall acceptability of drinking yoghurt samples. The product was developed according to the selected best formula and was subjected to analysis of chemical composition and shelf life evaluation. The sample containing 10% carrot pulp, 10% orange juice and 80% yoghurt base was selected as the best formula. Coliform, yeast and mold were absent after 35 days. The β -carotene content, antioxidant capacity and pH of the developed product were 0.495 ± 0.001 mg/100 g, $55.245 \pm 0.008\%$ and 4.416 ± 0.005 respectively. Collectively these results suggest that, the developed drinking yoghurt has 35 days of shelf life with high nutritional value including pro-vitamin A which is a natural antioxidant.

Keywords: β -carotene, vitamin A, natural antioxidant, drinking yoghurt, carrot pulp

1. Introduction

Milk is defined as a liquid, naturally secreted by the female mammary gland of mammals, for the nourishment of the young or offspring. According to Mourad and Bettache (2014), milk is the product of the total, full and uninterrupted milking of a dairy female in good health, also nourished and not overworked. It must be collected properly and not contain colostrum. Fermented or cultured dairy products are identified as the milk products produced by lactic acid fermentation (e.g. yoghurt) or a combination of lactic acid and yeast fermentation (Bylund, 1995). Yoghurt is a most popular fermented milk product which is consumed all over the world due to its sensory properties and high nutritional value. Yoghurt is offered in different types such as set, drinking, frozen, concentrated etc. considering the fat and total solid percentage, the body structure, and presence of additives, flavors or pulps with probiotic microflora. Further some bioactive compounds are used to fortify yoghurts (Srivastava et al., 2015). Generally, yoghurt drinks are flavored with natural or artificial fruit pieces or fruit juices according to the consumer preferences which is varied from country to country (Gunawardhana and Dilrukshi, 2016). Fruit additions have an increasing effect on yoghurt consumption (Farahat and El-Batawy, 2013).

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Carrot (*Daucus carota* L.) is a worldwide consumed vegetable with high economic importance (Xu et al., 2014) which is considered as a main source of carotenoid and pro-vitamin A (β carotene). The characteristic orange colour of carrots is given by carotenes (Suojala, 2000). β carotene is a natural antioxidant which is omnipresent in yellow and green vegetables and fruits. Within the body, β carotene is subjected to a metabolic pathway which forms vitamin A. Therefore β carotene is identified as pro-vitamin A and it is an important source of vitamin A (Keijer et al., 2005). It is a more efficient and abundant precursor of vitamin A. Inside the body, only a part of β carotene is converted to vitamin A; the rest is stored as β carotene. As a plant-based antioxidant, β carotene can control the excess formation of free radicals and increase the antioxidant capability as well as replace synthetic carcinogenic antioxidants which cause liver damage. The high dietary intake of carotenoids significantly increases the skin's endogenous level of UV photo protection by reducing its sensitivity to UV-induced erythema. And carotenoids specially β carotene can alter the skin color in short term (Alaluf et al., 2002). Therefore, it is more beneficial to consume carotenoid especially β carotene enriched food products. β carotene can be found in leaf, fruit, and even root tissues of many vegetable crops. The root crop carrot (*Daucus carota* L.) has some of the highest concentrations of β -carotene. β -carotene levels in carrot can range from 3.2 to 6.1 mg/100 g fresh weight (Kopsell and Kopsell, 2010).

Incorporation of plant-based antioxidants such as β carotene into dairy foods has met acceptance for the retardation of oxidation of dairy products. Further, natural antioxidants used to prolong the shelf life of the products as well as have fewer side effects than synthetic antioxidants to the human body (Alenisan et al., 2017). The rationale behind the research study is fortification of mostly consumed dairy product with β carotene as a solution for vitamin A deficiency in the world and further improvements for the effect of skin fairness. Therefore, the study was carried out to develop a dairy product; a drinking yoghurt by incorporating β carotene which is considered as a natural antioxidant abundant in most fruits and vegetables. The product was developed using Carrot as the main source of β carotene with the enhanced sensory properties of orange juice.

2. Materials and Methods

2.1 Development of drinking yoghurt with carrot pulp and orange juice

Standardized, homogenized, pasteurized, preheated cow milk was properly mixed with skimmed milk powder, sugar, gelatin, potassium sorbate and pasteurized at 95° C for 5 minutes. After reducing the temperature to 44° C, the yoghurt culture (*Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. bulgaricus*) was inoculated and incubated at 44° C until the pH was 4.5 and cooled to 4° C. Carrot pulp was obtained by blending grated carrot with water to 4:1 ratio. Orange juice was extracted; mixed with carrot pulp in different proportions (2:1, 1:1, 2:3) and pasteurized at 105° C for 1 minute. The three proportions of pasteurized carrot orange mix were mixed with prepared yoghurt mix while stirring into different proportions of yoghurt mix, carrot pulp and orange juice as formula A-17:1:2, formula B-16:2:2, formula C-15:3:2. The prepared drinking yoghurt samples were filled into bottles and stored at 4° C.

2.2 Sensory evaluation

A sensory trial was conducted in order to identify the best formula of yoghurt mix, carrot pulp and orange juice. Trained sensory panel with a nine-point hedonic scale was used for the sensory trial. The samples were coded as 253 for formula A, 426 for formula B, 389 for formula C and 528 for the control sample.

2.3 Analysis of chemical composition

The sample selected from sensory evaluation was subjected to analyse chemical composition. Moisture content, total solid content, total ash content were analysed according to the methods of Association of Official Analytical Chemists. The crude protein content was analysed using the Kjeldahl method, fat content was by Gerber method, total carbohydrate content was by Dubois method (Dubois et al., 1956). β carotene content was analysed by UV-Visible spectrometric method described by Rodriguez (2001), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was used to analyse the antioxidant activity of developed drinking yoghurt (Gjorgievski et al., 2013). The pH value of the sample was determined using pH meter (Mettler Toledo seven compact pH ion meter). The β carotene content, antioxidant activity and pH value of the sample which was stored at 4° C were analysed for 35 days with 7 days of intervals.

2.4 β Carotene content

Extraction of β carotene

About 5 g of drinking yoghurt sample was homogenized with 50 ml of cold acetone in an extraction tube. The repeated suction filtration was carried out in order to get all carotenoids extracted to acetone which is indicated by colourless/ white colour solid residue in the filter paper. For the separation, 20 ml of petroleum ether added separatory funnel was used. After incorporating acetone extract to the separatory funnel, 300 ml of distilled water was added by letting it flow along the walls of the funnel without shaking. The lower aqueous phase was discarded after the two phases were separated. The petroleum ether layer was washed out for 3-4 times by distilled water (200 ml for each time) to remove residual acetone and was collected while removing residual water using anhydrous Sodium Sulfate (15 g). The collected sample volume was measured and the procedure was triplicated for each sample.

Measurement of absorbance

The absorbance was measured using UV visible spectrophotometer (Thermo scientific-GENESYS 10S series) at the wavelength of 450 nm using quartz cuvettes. Petroleum ether was used as the solvent for calibrating the instrument to zero point. Three samples of each extract were used to measure the absorbance in order to get an accurate reading.

Calculation of β carotene content

The β carotene content (X) of samples was calculated using the following Equation (1) and (2).

$$X(\mu\text{g}) = \frac{\text{Absorbance at 450 nm} \times \text{Volume (ml)} \times 10^6}{\text{Absorption coefficient of } \beta \text{ carotene} \times 100} \quad (1)$$

$$X(\mu\text{g}) = \frac{X(\mu\text{g})}{\text{Weight of the sample (g)}} \quad (2)$$

Absorption coefficient of β carotene is 2592 (Rodriguez, 2001).

Antioxidant activity

Antioxidant activity of developed drinking yoghurt was determined according to a modified method of Gjorgievski et al., 2013.

Preparation of whey fraction from drinking yoghurt

Accurately measured 15 ml of the developed drinking yoghurt was poured into a centrifuge tube and centrifuged at 6,000 rpm for 10 minutes. The supernatant was filtered using a Whatman filter paper.

Preparation of DPPH solution

Accurately measured 3 mg of DPPH and the solution was prepared in a 50 ml volumetric flask using ethanol. The absorbance of the solution was measured using ethanol as the blank sample. The absorbance of the DPPH solution should be within 0.7-0.9 range. The prepared solution was covered and stored in a refrigerated condition.

DPPH radical scavenging activity

A volume of 2 ml of DPPH in ethanol was added to 2 ml of the whey fraction, mixed vigorously and allowed to stand in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. Ethanol was used as a blank, while DPPH solution in ethanol served as the control. The procedure was triplicated. The radical scavenging activity of the samples was expressed as % inhibition of DPPH absorbance:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad (3)$$

where:

A control = Absorbance of the control sample (DPPH solution without whey fraction)

A test = Absorbance of the test sample (DPPH solution plus whey fraction)

Microbial analysis

The microbial properties of developed drinking yoghurt; coliform count and yeast and mold count were evaluated according to standard methods. Violet Red Bile Agar medium was used to analyse coliform count and Yeast Dextrose Chloramphenicol Agar medium was used to evaluate yeast and mold count.

2.5 Statistical analysis

For the analysis of collected data, Minitab (17 version) statistical software package was used. The significant differences of mean values of each sample were analysed using One-way ANOVA at 95% confidence level. For the graphical representation of data Using Microsoft Office Excel 2016 package was used.

3. Results

3.1 Sensory analysis

Figure 1 shows the variation of mean values of each sensory attribute of the four drinking yoghurt samples (528-Control, 253-Formula A, 426-Formula B, 389-Formula C) according to the data gathered from sensory evaluation.

The mean values of each sensory attribute of Formula B are similar to the control sample except aroma. Other formulae are having lower values for sensory attributes than formula B and control. Therefore, formula B was selected as the best and subjected to product development and further analysis.

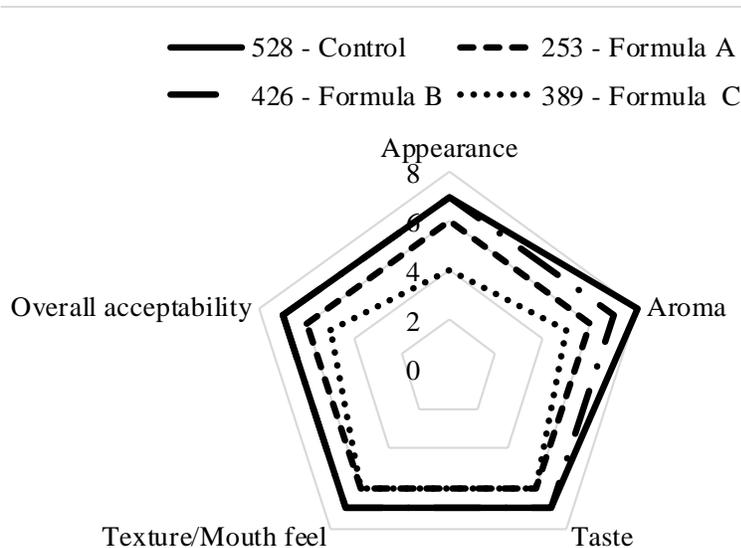


Figure 1. Graphical representation of selecting the best formula with respect to sensory attributes.

Table 1: H_{cal} values for each sensory attribute.

Attribute	H_{cal} value
Appearance	32.65
Aroma	19.38
Taste	26.76
Texture/Mouthfeel	32.28
Overall Acceptability	35.77

The degree of freedom of the test samples; $3-1=2$.

The H_{cal} values of each sensory attributes are greater than the relevant chi-square value (5.991). Therefore, according to the Kruskal Wallis test, under 0.05 level of significance, H_0 can be rejected. Which concludes that there is a significant difference in each sensory attributes of four samples.

3.2 Analysis of chemical composition

Table 2 summarizes the chemical composition of developed drinking yoghurt and plain drinking yoghurt. The moisture content of developed drinking yoghurt was $81.73 \pm 0.07\%$ with $18.27 \pm 0.07\%$ of the total solid. Total ash content was $0.58 \pm 0.05\%$, crude protein content was $3.16 \pm 0.10\%$, fat content was $2.2 \pm 0.1\%$, Total carbohydrate content was $4.59 \pm 0.18\%$ and β carotene content was $4.978 \pm 0.001 \mu\text{g/g}$.

Variation of pH value and β Carotene Content with storage at 4°C

Figure 2 indicates the graphical representation of the variation of pH value with storage at 4°C . The initial pH value of developed drinking yoghurt was 4.41 and reduced up to 4.20 within 35 days. The initial β carotene content of developed drinking yoghurt was $4.97 \mu\text{g/g}$. After 35 days of storage at 4°C , the β carotene content was reduced to $4.39 \mu\text{g/g}$ which is shown in Figure 3.

Table 2: Chemical composition of developed drinking yoghurt.

Component	Developed drinking yoghurt	Plain drinking yoghurt
Moisture	81.73±0.07%	83.43±0.05%
Total solid	18.27±0.07%	16.57±0.05%
Total ash	0.58±0.05%	0.67±0.00%
Crude protein	3.16±0.10%	3.69±0.05%
Fat	2.2±0.1%	4.05±0.05%
Total carbohydrate	4.59±0.18%	4.7±0.1%
β carotene	4.978±0.001 µg/g	0.428±0.001 µg/g

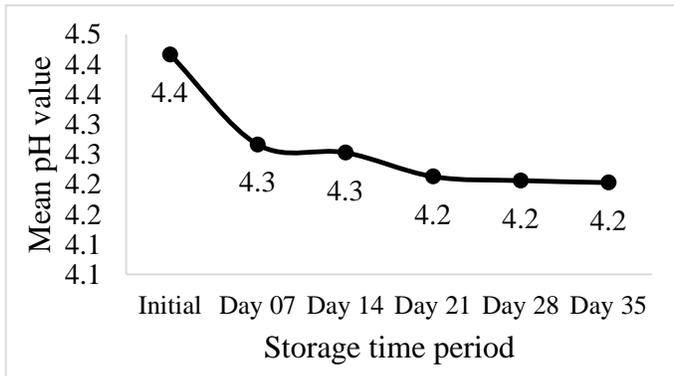


Figure 2. Variation of pH value with storage.

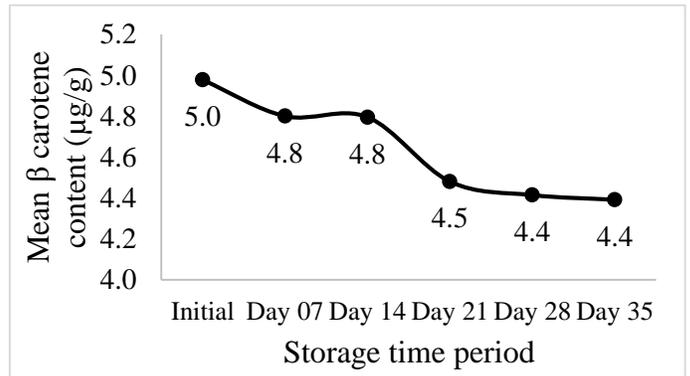


Figure 3. Variation of β carotene content with storage.

Variation of antioxidant activity with storage at 4° C

The antioxidant activity of developed drinking yoghurt was reduced from 55.2% to 38.3% within 35 days of storage time at 4° C as shown in Figure 4.

3.3 Microbial analysis

Microbial results are indicated in Table 3. Coliform counts were negative for 35 days in both developed sample and the control sample. Yeast and mold were also absent from the storage time period.

Table 3: Coliform, yeast and mold counts of the developed drinking yoghurt samples.

The time period of storage (at 4° C)	Coliform Count		Yeast and Mold count (CFU)	
	Developed drinking yoghurt sample	Control	Developed drinking yoghurt sample	Control
Initial	Negative	Negative	0	0
Day 07	Negative	Negative	0	0
Day 14	Negative	Negative	0	0
Day 21	Negative	Negative	0	0
Day 28	Negative	Negative	0	0
Day 35	Negative	Negative	0	0

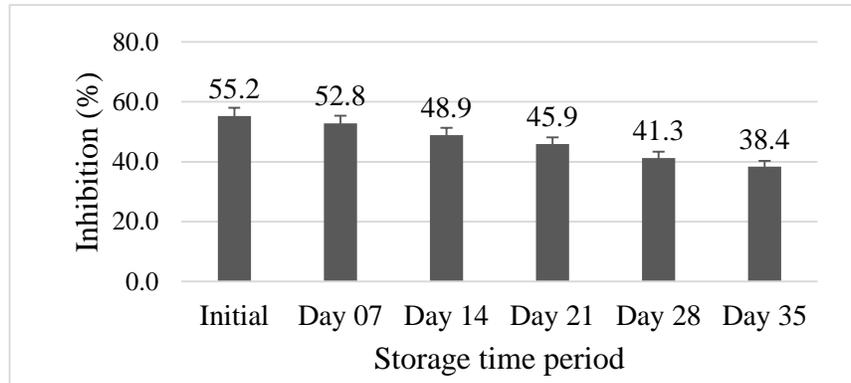


Figure 4. Variation of antioxidant activity with storage.

4. Discussion

The evaluated shelf-life of developed drinking yoghurt with 10% carrot pulp, 10% orange juice and 80% yoghurt base, was 35 days. Coliform and yeast and mold counts were negative and pH value was dropped to 4.20 from 4.41 within 35 days of the storage time period at 4° C. The moisture content of developed drinking yoghurt was $81.73 \pm 0.07\%$ with $18.27 \pm 0.07\%$ of the total solid. Total ash, crude protein, fat, total carbohydrate and β carotene contents were respectively $0.58 \pm 0.05\%$, $3.16 \pm 0.10\%$, $2.2 \pm 0.1\%$, $4.59 \pm 0.18\%$ and $4.978 \pm 0.001 \mu\text{g/g}$. The developed drinking yoghurt had a higher amount of β carotene when compared with the plain drinking yoghurt.

A drinking yoghurt incorporated carrot juice with three weeks of shelf life has developed by Salwa et al. (2014) which reveals that 15% carrot juice is the formula having the highest consumer acceptance selected from the formulae of 5%, 10%, 15% and 20% carrot juice while the formula with 10% carrot pulp is having the highest consumer acceptance in the current study. According to the study, the growth of coliform and yeast & mold have been suppressed by high amount of carrot juice which reveals that carrot is having significant effect on the acceptability of yoghurt during shelf life. The short shelf-life of carrot juice due to the low acidity, which is favourable for microbial growth, limits the storage life of carrot juice which limits the benefits of β carotene (Beveridge, 2002; Sinchaipanit and Kerr, 2007). Therefore, to extend the shelf life of carrot juice, improve its quality and marketability, increasing its acidity could be an effective method (Demir et al., 2007) which can be obtained from incorporating carrot juice into yoghurt like acidic product with citrus fruit juice. In general, the most commonly used methods of acidification of vegetable juices include: adding organic acid, such as citric acid (Reiter et al., 2003; Sharma et al., 2009) and acetic acid (Shivhare et al., 2009); the use of acid-producing bacteria (Demir et al., 2007; Wiander and Korhonen, 2011) and blending with acidic fruit juices such as orange, lemon, apple and cranberry juice (Selma et al., 2004). Therefore, incorporation of carrot pulp with orange juice for a drinking yoghurt leads to extend the shelf life of carrot juice while increasing the beneficial effects of β carotene.

A drinking yoghurt incorporated avocado pulp has been developed by Gunawardhana and Dilrukshi (2016), which is containing 3.0-3.2% fat with 4.41 ± 0.02 to 4.12 ± 0.05 of pH value which is similar as in the current study. Incorporating natural fruit and vegetable pulps to yoghurt enhance consumer acceptance while increasing its nutritional value and shelf life.

A research study carried out by Gad et al. (2015), reported that a 8% carrot juice incorporated yoghurt with 12 days of shelf life which contains about 1,400 ($\mu\text{g}/100 \text{ g}$) total carotenoids equiv. β

carotene; a type of carotenoid which is also known as pro-vitamin A is a natural antioxidant mostly present in fruits and vegetables. For foods supplements, options are to limit the use of β carotene to either 2 mg/day, or to 2-4 mg/day, which is the desirable intake according to the German Nutrition Society. For fortified foods, suggested options are either not to allow β carotene for food fortification at all, or limit the use to 2 mg/100 g or 100 ml (Strobel et al., 2007). Since the drinking yoghurt developed in the current study is containing $4.978 \pm 0.001 \mu\text{g/g}$ of β carotene, 400 g of particular drinking yoghurt can fulfill the daily requirement of β carotene.

A fortified yoghurt with red ginseng extract has been developed by Jung et al. (2016), which is having higher antioxidant activity (89.44-94.80%) compared to the drinking yoghurt developed in the current research study (38.36-55.24%). Similarly, a gradually decreasing antioxidant activity within 21 days is observed in a drinking yoghurt incorporated green tea extract which has been developed by Thomas and Wansapala (2017). The studies carried out by Senadeera et al. (2018), and Nguyen and Hwang (2016) also justify that, the antioxidant activity of drinking yoghurts incorporated natural fruit pulps is reduced with the storage at the refrigerated condition which limiting the action of β carotene as a powerful antioxidant for preventing tumor growth, enhancing immune response and inhibiting mutagenesis (Pritika, 2015).

Future recommendations of the study are to analyse the consumer behavior towards developed drinking yoghurt using consumer panels which may lead to a market analysis of developed drinking yoghurt and to detect the skin fairness effect of β carotene and developed drinking yoghurt through an in vivo analysis as the two most prominent carotenoids in the skin are β -carotene and lycopene.

This study discovered β carotene enriched drinking yoghurt incorporated with carrot pulp and orange juice that can be beneficial for vitamin A deficiency and skin fairness of humans. This study will help the researchers to uncover the critical areas of β carotene stability and its skin fairness effect that many researchers were not able to explore. Thus a new theory on β carotene fortified dairy products for both vitamin A deficiency and skin fairness may be arrived at.

5. Conclusion

The best formulation of drinking yoghurt was 80% yoghurt mix, 10% carrot pulp and 10% orange juice with respect to the sensory attributes.

The shelf life of the developed drinking yoghurt was 35 days (5 weeks). In the developed drinking yoghurt, coliform counts were negative and yeast and mold counts were zero within 35 days of storage time at 4° C. The pH value, β carotene content and antioxidant activity are reduced with storage time at 4° C. The β carotene content of developed drinking yoghurt was 11.63-times higher than plain yoghurt.

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