

Effect of Baked, Whipped and Fermentation on Antioxidant Activity in Red Raspberries

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Abstract

Red raspberries (*Rubus idaeus*) are a good source of antioxidants and contains appreciable levels of phenolic compounds (TPC). Adding raspberry to the product are attributed the most significant health benefits of to the phenolic compounds. This study examined the three different manufacturing processes baked, whipped and fermentation on antioxidant activity in red raspberry. The phenolic compounds in red raspberry, sponge cake, whipping cream and yoghurt by red raspberry were determined by HPLC. Sensory evaluation found that the best proportions to add red raspberry to whipped cream and yogurt is 10% but in the sponge cake is 15%. The total phenols were 56%, 37% and 4%, 3% of red raspberry, red raspberry-yoghurt, red raspberry-whipped cream and red raspberry-sponge cake respectively. So the treatments were order in general to their effect of the TPC: fermentation > whipped > baked.

Keywords: Antioxidant; Baked; Fermentation; Red raspberry; Whipped

Introduction

Recently, Plants of the genus *Rubus* (family: Rosaceae) have been reported to exhibit several biological activities such as anti-diabetic, anti-oxidative, anti-inflammatory and anti-hyperlipidemic activities. These biological activities were due to their polyphenol components including anthocyanins present in some of the varieties [1]. Red raspberries (*Rubus idaeus*) are among the fruits containing the highest antioxidant levels. In addition to vitamin C, the antioxidant activity of red raspberries is primarily constituted by two classes of compounds: anthocyanins and ellagitannins. Ellagitannins, which are complex derivatives of ellagic acid [2], have been identified in tea, many medicinal plants, and several fruits, including raspberries [3,4]. In addition to their vasorelaxation properties [5], ellagitannins have been described to have general antioxidant effects [6]. Red raspberry could therefore be considered as a model fruit source for a variety of potentially healthy compounds [7]. Berries, fruits full of bioactive compounds, are also very delicious, have low energy [8]. To the bioactive compounds group in berries belong antioxidants such as phenolic compounds and fruit colorants (anthocyanins and carotenoids). Berries' phenolics represent a diverse group of compounds including phenolic acids, such as hydroxybenzoic and hydroxycinnamic acid conjugates; flavonoids, such as flavonols, flavanols, and anthocyanins. In addition, tannins, divided into condensed tannins (proanthocyanidins) and hydrolyzable tannins, are reported to be important bioactive compounds. These compounds are of great interest for nutritionists and food technologists due to the opportunity to use bioactive compounds as functional foods ingredients. Nutraceuticals and functional foods have become very popular for people due to the consumer demands for healthy nutraceutical foods that could possibly reduce some health risks and improve various health conditions [9]. The anthocyanins are stable at low temperatures and in the dark [10]. The highest temperature combined with a short baking time had the best effect on the preservation of polyphenols, in order to achieve the most favorable nutritional effect of baked products enriched with fruit pomace [11]. pH is closely related to microbial growth and the structural changes in phytochemicals during fermentation. For example, anthocyanin breakdown is dependent on the pH in the presence of oxygen, is also directly related to the level of pseudo base, and is inversely related to the cation concentration [12]. Several studies [13,14] have shown that

anthocyanins are stable at low pH. Anthocyanins exhibit the highest stability, with the red flavylium cation stable around pH 1-2 [14]. The stability of anthocyanins is dependent on their structure; for instance, acylated anthocyanins are more stable than the non-acylated forms [15]. pH is a dominant factor in the radical scavenging capacity of wine anthocyanins, as an increase in pH often increases the capacity for radical scavenging [16]. Colour plays a very important role in the acceptability of some foods by many consumers [17]. In practice, most manufacturers tend to colour products which have dull colours and look unappealing to most consumers. Synthetic colourants have often been used in attempts to colour some foods and beverages [18]. However, the demand for foods with synthetic anthocyanins are a great interest as alternatives to synthetic colourants due to their bright colours and associated health benefits [19,20]. They are considered to be safe because they have been consumed for centuries in fruits, and vegetables without any health risks [21]. Whole fruit extracts containing non-acylated anthocyanins from *Berberis boliviana* L. showed improved colour and pigment stability when incorporated in yoghurt [22]. The anthocyanins are stable at low temperatures and in the dark [10] For that we believe that whole fruit juice extracts from red raspberries (*Rubus idaeus*) could serve as an appropriate colorant and nutraceutical in yoghurt. Raspberry fruits are rich in phenolic compounds contents such as phenolic acids [23,24] flavonoids [24,25] and anthocyanins [23]. The phenolic compounds in berries have been reported to have antioxidant, anticancer, antiinflammatory, and antineurodegenerative biological properties [26,27]. In recent years, red raspberry anthocyanins have in many occasions been applied in baked foods, and confectioneries [28]. In this study, we decided to investigate whether anthocyanins from Red raspberry (*Rubus idaeus*)

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Received August 30, 2016; Accepted September 17, 2016; Published September 23, 2016

Citation: Darwish AZ, Bayomy H, Rozan M (2016) Effect of Baked, Whipped and Fermentation on Antioxidant Activity in Red Raspberries. J Food Process Technol 7: 621. doi: 10.4172/2157-7110.1000621

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could be used as potential colour additives in yoghurt since yoghurt has a low pH and it is stored under refrigerated conditions. Therefore, the current study was undertaken to use the red raspberries (*Rubus idaeus*) in industry of yoghurt, cake and the formation of cream as colorant. And study the effect of these processes on the antioxidant activity.

Methods

Materials

Whipping cream from the brand Almarai (Kingdom of Saudi Arabia) was purchased from the local supermarket. This is an ultrahigh temperature (UHT) product containing 33% milk fat, 1.9% protein and 3.5% carbohydrate. Cream was kept in fridge at 5°C or below during storage. Commercial wheat flour was purchased from Kuwait Flour mills & Bakeries Co. (Kuwait). Sunflower oil, sucrose, batter and fresh eggs were purchased from local market in Tabuk, Kingdom of Saudi Arabia.

Sponge cake preparation

The sponge cakes were prepared according to Chaiya and Pongsawatmanit [29]. The experiments used sponge cake batter formulations containing WF (50-100 g), 140 g liquid whole eggs, 10 g whole milk powder, 2 g baking powder, 120 g sugar, 80 g butter and 40 g water. In the cake batter preparation (~500 g), the liquid whole eggs, water, sugar were mixed in a using Kenwood-kitchen machine 1200 W (China) with machine speeds from 1 to 10 at speed 3 for 1 min and further mixed at speed 6 for 9 min. Then, dry ingredients (the flour blend of WF, whole milk powder and baking powder) were added simultaneously to the mixture at speed 1 for 1 min and further mixed at speed 3 for 2 min. The melted butter was added finally and mixed at speed 1 for 20 s. The batter was divided into four portions formulation each one 125 g (control and three with red raspberry puree as following: red raspberry spongy cake 10% (12.5 g), red raspberry spongy cake 15% (18.75 g) and red raspberry spongy cake 25% (31.25 g). Each one was placed in pan (8.5 × 16 × 5 cm) and baked in oven at 175°C for 20 min. After baking, the cakes were removed from the pans, cooled upside down on a wire rack for 30 min at room temperature and kept in plastic bags to prevent drying before being measured for sensory evaluation within 12 h.

Whipped cream preparing

Cream must be kept in fridge at least 24 h before tempering. In each experiment, 100 ml cream was mixed with different amount of raspberry puree (5, 10 and 15 g) using Kenwood-kitchen machine 1200 W (China).

Yoghurt preparing

Yoghurt was manufactured using lactobacillus delbrueckii subsp bulgaricus and streptococcus salivarius subsp thermophilus (1:1) commercial starter culture. Fresh cow's milk was heated to 90°C for 10 min, and cooled to 42°C. Milk was mixed with different percentage of red raspberry (5, 10 and 15%) after that adding 3% starter and incubated at same temperature.

Proximate analysis

Materials (red raspberry puree, cream, yoghurt and sponge cake) were dried at 70°C to a constant weight, moisture contents, ash, and crude fiber were determined by AOAC [30] methods. Total lipids from the samples were extracted with chloroform/methanol (2:1, v/v) and quantified gravimetrically [31]. Nitrogen content (N) of the sample was estimated by the method described by Kjeldahl [32] and crude protein

was calculated as $N \times 6.25$ [33]. The amount of total carbohydrates was obtained by the difference between weight of the sample taken and sum of its moisture, ash, total lipid, protein, and fiber contents [34].

Analysis of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure [35]. Briefly, the extract (500 µl) was transferred into a test tube and oxidized with the addition of 250 µl of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na_2CO_3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as µg of gallic acid equivalent (GAE) per ml of sample.

Analysis of total flavonoid content

The total flavonoid content was determined according to Zilic et al. [35]. Briefly, 250 µl of 5% NaNO_2 was mixed with 500 µl of extract. After 6 min, 2.5 ml of a 10% AlCl_3 solution was added. After 7 min, 1.25 ml of 1 M NaOH was added, and the mixture was centrifuged at 5000 g for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as µg of catechin equivalent (CE) per ml of sample.

Determination of radical DPPH scavenging activity

Free radical scavenging capacity was determined using the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH[•]) according to Hwang and Thi [36]. The final concentration was 50 µM for DPPH and the final reaction volume was 3.0 ml. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

Where

A_{control} is the absorbance of the control reaction (containing all reagents except the test compound).

A_{sample} is the absorbance of the test compound. Also, the antioxidant activity was determined by means of a calibration curve prepared with Trolox, and expressed as mg of Trolox equivalent (TE) per unit (volume or weight) of sample.

Phenolic acids profile

Extraction of phenolic compounds: The sample was alkaline hydrolyzed according to Kim et al. [37]. Sample (1 g) was placed in quick fit conical flask and 20 ml of 2 M NaOH was added and the flasks were flushed with N_2 and the stopper was replaced. The samples were shaken for 4 h at room temperature. The pH was adjusted to 2 with 6 M HCl. The samples were centrifuged at 5000 rpm for 10 min and the supernatant was collected. Phenolic compounds were extracted twice with 50 ml ethyl ether and ethyl acetate 1:1. The organic phase was separated and evaporated at 45°C and the samples redissolved in 2 ml methanol.

Analysis of phenolic compounds by HPLC: HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 × 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total

run time of 70 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Sensory evaluation

The hedonic test was used to determine the degree of overall liking for the whipped cream, sponge cake and yogurt. For this study, untrained consumers were recruited from the students, staff. All consumers were interested volunteers and informed that they would be evaluating whipped cream, sponge cake and yogurt. 15 consumers (7 males and 8 females, 19-55 years) received samples were asked to rate them based on degree of liking on a seven-point hedonic scale (1 = dislike extremely, 4 = neither like nor dislike, 7 = like extremely). Samples were placed on white plates and identified with random numbers. Panelists evaluated the samples in a testing area and were instructed to rinse their mouths with water between samples to minimize any residual effect [38]. Where the evaluation in terms of color, taste and smell and textures in addition to the overall acceptance.

Statistical analysis

Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using SPSS version 9.0 program to examine statistical significance differences of sensory analysis means of experimental data. Results were considered statistically significant when $p < 0.05$. Mean \pm standard deviation values were also presented.

Results and Discussion

Sensory evaluation

Results of sensory evaluation for reach to the best proportions to add red raspberry to whipped cream, sponge cake and yogurt are reported in Table 1. When evaluated by untrained consumers, statistically significant differences were detected in all of the sensory attributes evaluated ($P \leq 0.05$). It is clear that the best proportions to add red raspberry to whipped cream and yogurt is 10% but in the sponge

cake is 15%. With regard to color, taste, smell texture and the overall acceptance 10% red raspberry whipped cream, 10% red raspberry yogurt and 15% red raspberry sponge cake were appreciated the most significantly higher preference scores than the other treatments ($P \leq 0.05$).

Proximate composition

Table 2 describes the proximate composition of red raspberry puree and foods that have been selected from the sensory evaluation, which have ratios of red raspberry. These foods include whipped cream (with 10% red raspberry), yoghurt (with 10% red raspberry) and sponge cake (with 15% red raspberry).

Total phenolic content

The total phenolic content (TPC) for sponge cake, yoghurt and whipped cream (Table 3). Highest TPC in samples was found in sponge cake (0.709 ± 0.08 mg GEA/ml) which were hated at 175°C for 20 min. that may case decreasing percentage in TPC than red raspberry because though heat-treated lowered the antioxidant level, and adding ingredients such as sugar diluted the antioxidant concentration, products made from berries are high sources of antioxidants [39-41]. The highest value compare with yogurt and whipped cream may be due to the production of Maillard reaction products in the crust during thermal processing [42]. Similar observations have been made when baking rhubarb, whereby both TPC and FRAP AA were higher during the first 20 min and then decreased to low levels [43] and when baking chocolate cookies and chocolate cakes made with baking powder rather than baking soda [44]. TPC in red raspberry-whipped cream was 0.081 ± 0.004 mg GEA/ml decreasing percentage in TPC this may be due to whipped processes. Yogurt has recorded the lowest content of TPC 0.067 ± 0.001 mg GEA/ml. During fermentation process microbial yoghurt utilization of phenolic acids such as ferulic and p-coumaric acid and post acidification lead to the production of other phenolic acids such as vanillic and p-hydroxybenzoic acids before the aromatic ring structure is broken down [45]. Also the decreasing of TPC than the red raspberry there were increasing in the TPC red raspberry-yogurts than plain-yoghurt 0.008 mg GEA/ml that can be explained by the presence of indigenous phytochemical compounds in raspberry (e.g., flavonoids and phenolic compounds) [46]. The major TPC were 63.929 ± 3.000 , 46.162 ± 5.100 , 38.617 ± 5.000 and 11.320 ± 1.000 µg/g of gallic

	Whipped cream			Sponge cake			Yogurt		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
Color	7.4 \pm 0.06 ^c	9.4 \pm 0.02 ^a	8.3 \pm 0.03 ^b	7.3 \pm 0.04 ^b	7.4 \pm 0.05 ^b	8.7 \pm 0.04 ^a	5.7 \pm 0.06 ^c	8.8 \pm 0.03 ^a	7.1 \pm 0.01 ^b
Taste	8.3 \pm 0.02 ^b	9.2 \pm 0.02 ^a	8.7 \pm 0.05 ^b	6.3 \pm 0.07 ^a	6.8 \pm 0.07 ^a	6.5 \pm 0.07 ^a	8.4 \pm 0.03 ^b	9.5 \pm 0.02 ^a	9.5 \pm 0.02 ^a
Smell	8.0 \pm 0.04 ^b	9.3 \pm 0.03 ^a	9.2 \pm 0.03 ^a	6.1 \pm 0.05 ^b	6.3 \pm 0.03 ^b	8.7 \pm 0.06 ^a	7.6 \pm 0.04 ^b	8.4 \pm 0.04 ^a	8.7 \pm 0.02 ^a
Texture	7.9 \pm 0.04 ^a	7.4 \pm 0.04 ^a	6.3 \pm 0.07 ^c	8.9 \pm 0.03 ^a	8.3 \pm 0.05 ^a	8.2 \pm 0.02 ^a	9.2 \pm 0.03 ^a	9.4 \pm 0.02 ^a	8.3 \pm 0.05 ^b
Overall acceptance	8.5 \pm 0.03 ^a	9.2 \pm 0.04 ^a	8.6 \pm 0.04 ^a	7.8 \pm 0.03 ^a	7.8 \pm 0.01 ^a	8.4 \pm 0.03 ^a	7.6 \pm 0.05 ^c	9.1 \pm 0.03 ^a	8.5 \pm 0.04 ^b

Means within the same row without a common letter (a-c) are significantly different ($P \leq 0.05$) for each type.

Table 1: Sensory evaluation for red raspberry-whipped cream, red raspberry-sponge cake and red raspberry-yogurt.

Proximate composition (g/100 g FW)	Red raspberry fruit	Whipped cream	Yoghurt	Sponge cake
Moisture	81.43 \pm 2.06	61.53 \pm 2.58	87.71 \pm 2.73	35.82 \pm 1.06
Ash	0.51 \pm 0.08	0.14 \pm 0.07	0.79 \pm 0.08	1.22 \pm 0.14
Lipid	0.57 \pm 0.12	33.07 \pm 1.07	3.26 \pm 0.22	19.6 \pm 0.77
Protein	1.49 \pm 0.24	1.64 \pm 0.32	3.42 \pm 0.41	5.95 \pm 0.30
Fiber	1.59 \pm 0.68	0.23 \pm 0.04	0.21 \pm 0.09	0.87 \pm 0.06
TC	14.41 \pm 1.63	3.39 \pm 0.24	4.69 \pm 0.38	36.54 \pm 1.21

FW: Fresh Weight; DW: Dry Weight; TC: Total Carbohydrates; Values (Mean \pm SD)

Table 2: Proximate composition of red raspberry, whipped cream (with 10% red raspberry), yoghurt (with 10% red raspberry) and sponge cake (with 15% red raspberry).

Compound(ug/g)	Red raspberry	Sponge cake	Whipped cream	Yoghurt
Gallic acid	63.929 ± 3.000	35.497 ± 4.000	19.365 ± 2.000	16.996 ± 2.000
Protochatchuic acid	11.320 ± 1.000	4.814 ± 0.300	17.172 ± 5.300	28.343 ± 5.300
Gentisic acid	0.965 ± 0.070	ND	ND	ND
Catachine	46.162 ± 5.100	34.079 ± 6.000	37.875 ± 6.200	31.966 ± 4.000
Chlorogenic acid	ND	ND	ND	ND
Caffeic acid	4.073 ± 0.400	3.511 ± 0.200	2.458 ± 0.100	2.156 ± 0.300
Syrngic acid	ND	0.467 ± 0.030	ND	ND
Vanillic acid	1.832 ± 0.100	22.803 ± 0.300	2.938 ± 0.100	0.907 ± 0.400
Rutin	ND	ND	ND	ND
Coumarin	38.617 ± 5.000	29.571 ± 0.300	12.032 ± 1.00	10.680 ± 1.000
Ferulic acid	8.342 ± 0.300	1.061 ± 0.100	2.991 ± 0.002	2.058 ± 0.003
Sinapic acid	4.722 ± 0.006	3.094 ± 0.300	1.453 ± 0.001	1.542 ± 0.001
Rosmarinic acid	2.150 ± 0.003	0.751 ± 0.006	ND	0.569 ± 0.004
Cinnamic acid	1.274 ± 0.004	1.334 ± 0.400	0.530 ± 0.003	0.582 ± 0.002
Qurecetin	0.537 ± 0.003	ND	0.499 ± 0.003	0.169 ± 0.003
Kaempferol	0.709 ± 0.005	0.398 ± 0.002	2.717 ± 0.3	0.189 ± 0.001
Chyrsin	3.647 ± 0.004	3.075 ± 0.200	4.873 ± 0.300	2.090 ± 0.200

Mean ± standard deviation (n=3).

Table 3: Contents of main phenolic compounds of red raspberry, raspberry-sponge cake, raspberry-whipped cream and raspberry-yogurt by HPLC.

Sample	Total phenols (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH (mg TE/g)
Red raspberry	1.088 ± 0.02	0.216 ± 0.003	2.270 ± 0.5
Yoghurt	0.067 ± 0.05	0.026 ± 0.001	0.289 ± 0.07
Whipped cream	0.081 ± 0.004	0.069 ± 0.001	0.353 ± 0.002
Sponge cake	0.709 ± 0.08	0.151 ± 0.004	0.591 ± 0.03

Mean ± standard deviation (n=3).

Table 4: Total phenols, total flavonoids and DPPH of red raspberry, raspberry-sponge cake, raspberry-whipped cream and raspberry-yogurt.

acid, catachine, coumarin and protochatchuic acid, respectively in the red raspberry. On the other hand, gallic acid, catachine, coumarin and vanillic acid were recorded 35.497 ± 4.000, 34.079 ± 6.000, 29.571 ± 0.300 and 22.803 ± 0.300ug/g respectively in the red raspberry- sponge cake. The higher TPC in the red raspberry-whipped cream were 37.875 ± 6.200, 19.365 ± 2.000 and 17.172 ± 5.300 and 12.032 ± 1.00 ug/g of catachine, gallic acid, protochatchuic acid and coumarin, respectively. The highest TPC found in red raspberry-yoghurt were 31.966 ± 4.000 28,343 ± 5.300, 16.996 ± 2.000 and 10.680 ± 1.000 ug/g of catachine, protochatchuic acid, gallic acid and coumarin, respectively. Adding raspberry to the product are attributed the most significant health benefits of to the phenolic compounds, such as flavonoids, phenolic acids [47]. So the treatments were order in general to their effect of the TPC: fermentation > whipped > baked.

DPPH scavenging activity

Red raspberry-sponge cake had higher antioxidant activity than red raspberry-whipped cream and raspberry-yogurt (Table 3). Highest DPPH in red raspberry-sponge cake than in red raspberry -whipped cream and red raspberry-yogurt were most likely contributed by individual phytochemical contents and as a result of microbial metabolic activities [48]. The lowest in yoghurt may be due to attributed to the metabolically active yogurt bacteria [49]. High antioxidant activities useful for protective cardiovascular effect [50]. The DPPH radical-scavenging highest with heat followed by whipped and fermentation. The DPPH radical-scavenging activity was 0.591, 0.353 and 0.289 mg TE/g of red raspberry-sponge cake, red raspberry-whipped cream and red raspberry-yogurt respectively (Table 4).

Total flavonoids

Table 2 shows that the baking treatment had higher total flavonoids than the whipping and fermentation. The mean values of red raspberry

were 0.216 ± 0.003 mg CE/g followed by red raspberry-sponge cake value 0.151 ± 0.004 mg CE/g and red raspberry-whipped cream 0.069 ± 0.001 mg CE/g, while yoghurt was recorded lowest value 0.026 ± 0.001 mg CE/g. flavonoid compounds of raspberry have significant antioxidant activities consumption may help prevent and/or moderate chronic diseases these antioxidant properties and health benefits and for tailored breeding for functional foods [51].

Conclusion

Red raspberry sponge cake could be further used as a source of natural antioxidants for application in the nutraceutical or functional food areas. Much higher losses of total phenols were found in baked, whipped and fermentation treatment. A strong effect of treatment on phenols content was found in fermentation treatment. The products produced with red raspberry contained 56%, 37% and 4%, 3% of raspberry total phenols of red raspberry, red raspberry -yoghurt, red raspberry -whipped cream and red raspberry-sponge cake.

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