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The Radicals Scavenging Activity of Yogurt Fortified with Rose Hip Marmalade During 21 Days of Storage Period

Rola Alzamara [1*] Talha Demirci [2] Nihat Akin [3]

1. Selcuk University, Faculty of Agriculture, Department of Food Engineering, Konya 42030, Turkiye.
2. Selcuk University, Faculty of Agriculture, Department of Food Engineering, Konya 42030, Turkiye.
3. Selcuk University, Faculty of Agriculture, Department of Food Engineering, Konya 42030, Turkiye.

* E-mail of the corresponding author: Rola.Alzamara@yahoo.com

Abstract

In this study, the handmade rose hip marmalade was added to regular yogurt (before or after fermentation) and in different concentrations (5, 10, 15%). The physico-chemical, micro-biological, antioxidant activity and phenolic compounds properties of yogurt fortified with rose hip marmalade were determined during 21 days of storage period. Practical experiments show that the rose hip marmalade participate in enhancing the radical scavenging activity, phenolic compounds and water holding capacity of yogurt. While gradually the conditions and duration of storage contribute to significant decreases in radical scavenging activity, phenolic compounds and water holding capacity as well as the sensory evaluation. Results show that the 15% concentration of rose hip marmalade increased the radical scavenging activity in comparing with other concentrations. On the other hand the best effect of marmalade concentrations in overall properties was recorded in 10% concentration; also the shelf life of yogurt fortified with rose hip marmalade was determined in 14 days.

Keywords: antioxidant, radical scavenging activity, free radicals, rose hip marmalade, yogurt.

1. Introduction

The nutritive value of mature dog rose hip fruit (Cynosbati fructus) not restricted on vitamin C but also to vitamins (B1, B2, K, PP, D, and E), sugars, organic acids, pectins, flavonoids, tannins, carotenoids (β-carotene, licopene, and isomeres of rubixanthin), macro and microelements, …etc (Pârvu, 2000; Demir and Ozcan, 2001; Tiţă, 2003; Stănescu et al., 2004; Arsenescu, 2008; Orhan et al., 2009). The most common use of rose hip varieties throughout history has been for prevention and treatment of colds and flu where the rose hip content from vitamin C was estimated as more than 30-50 times from oranges content (Ercisli and Esitken, 2004). These super antioxidants complement vitamin C and enhance its effect on the body by neutralizing the free radicals which is produced in the oxidative processes (Yi, 2007).

The imbalance resulting of increasing the products of oxidative processes, like reactive oxygen species and reactive nitrogen species, causes the decreasing of antioxidant sources, which leads to many degenerative diseases such as cancer, cardiovascular disease and age-related diseases (Ygi, 1987; Cutler, 1984-1992).

Biologically antioxidant is defined as any molecule capable of deactivation or stabilizing free radicals to protecting the body against oxidative damage and diseases, in contrast the Food and Drug Administration (FDA) defined antioxidants only as dietary supplements to be taken in addition to normal food consumption in an effort to prevent diseases. Antioxidants can also be used as a form of preservatives to maintain freshness and extend shelf lives of foods.

Generally antioxidants are divided into natural and synthetic depending on the source. Natural antioxidants are present in natural sources such as fruits and vegetables. On the other hand the synthetic antioxidants are synthesized chemically such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT). It has been shown that the artificial sources compounds have carcinogenic side effects, by causing oxidative damage to DNA (Gould 1995; Chun et al. 2006).

In recent years, several researchers have attempted to fortify the poor food sources of antioxidants with rich natural sources antioxidants such as fortifying yogurt with rich natural sources antioxidants. The combining of the original nutrients of yogurt, beneficial effects of starter cultures and rich natural sources of antioxidants, makes it more convenient food format to satisfy consumers (Chouchouli 2013). Fortifying yogurt with different types of fruits (peach, orange, lemons, purple plum, boysenberry, strawberry, raspberry, blueberry and cherry)

and in different forms (jams, marmalade, fruit jellies, fruits drinks, fruit syrups and concentrated fruit) works as a good source of antioxidants (Chandan et al. 1993, 2006). Several studies talks about the secondary metabolites which are synthesised by plants during normal development or as a responses to environmental stress, such as polyphenols. These secondary metabolites have antioxidants activities that act as free radical scavengers, electron or hydrogen donors or strong metal chelators. These leads to preventing oxidations and thereby considered as a preventer agents against several degenerative diseases (Pezzuto 2008; Williamson and Manach 2005).

This present study is designed to prepare rose hip marmalade yogurt (RHMY) by fortifying yogurt with different amounts of rose hip marmalade (RHM) (5, 10 and 15%), by performing this before and after fermentation. The study also aims to determine the effect of rose hip marmalade (RHM) on yogurt properties during 21 days of storage period. Rose hip marmalade considered as a rich resource of high antioxidant and nutritive compounds.

2. Materials and Methods

2.1 Rose hip marmalade preparation

The rose hip fruit was collected from Konya region in Turkey. To prepare marmalade 50% of fruit was mixed with 50% of sugar, the mix was boiled for 5 minutes and was filled directly into clean glass jars. Marmalade was stored in a room temperature until usage in yogurt production.

2.2 Yogurt preparation

Yogurt was prepared according to Tamime and Robinson (1985). The standardized and pasteurized milk was taken from ENKA dairy factory in Konya/Turkey. The milk was heated to 44±1°C and was inoculated with 2% commercial freeze-dried yogurts starter cultures (S. thermophilus and L. d. bulgaricus) (Chr's Hansen-Peyma Istanbul). The inoculated yogurt milk was divided into four portions and was incubated at 43±1 °C until pH 4.6, after that it was cooled to 10°C. One portion from the cooled yogurt was taken as control, the other portions were fortified with different percentages from RHM (5, 10 and 15%) as a stirred yogurt. For set marmalade yogurt the different percentages from RHM marmalade were added before fermentation. All yogurt was stored at 4°C for further analysis.

2.3 pH and titratable acidity

The pH was determined after calibrating the pH meter (WTW 315i Set brand) with buffer standards of pH 4.01 and pH 7.0. 20ml was taken from diluted sample (1:1, yogurt: distilled water) and was stirred uniformly about 2-3 minutes, after that the sample was measured by inserting probe of the pH meter into the sample. The probe was rinsed thoroughly with distilled water before usage in sample (Metin 2008). Titratable acidity of the yogurt samples was determined according to the standard AOAC procedure (Helrich 1990).

2.4 Syneresis and water holding capacity (WHC)

Syneresis of analyzed yogurt was determined by putting filter paper on the top of a funnel, after that it was spread approximately 100g from the sample on the paper. The drainage time and temperature degree was 2h at 4˚C. The percentage of syneresis was calculated as (liquid weight/initial sample weight) x 100 (Farooq and Haque 1992). For water holding capacity (WHC), approximately 10g of yogurt was placed in test tube and centrifuged at 5000rpm for 20 minutes at 4˚C, the WHC was expressed as (clear supernatant/ initial weight) x 100. Each treatment was replicated 3 times (Çelik and Bakırcı 2003).

2.5 Extraction of yogurt samples

The samples were measured after water had been extracted from yogurt. For this purpose, 3g yogurt was mixed with suitable amount (30ml) of methanol dilution (80:20, methanol: distilled water). After that the mix was homogenized by ultra-turrax homogenizer and centrifuged to 7200 rpm for 10 min at 24°C. The filtered liquid portion through (Whatman No.1) was stored at 4˚C for antioxidant activity analysis.

2.6 Total phenolic content assay (TPCs)

The total phenolic compounds in yogurt were determined by using the modified method of Shetty et al. (2005).

One ml of yogurt sample (1ml) was mixed with 1.0 ml of 95% ethanol and 5ml of distilled water. After that 0.5 ml Folin-Ciocalteu diluted reagent (1:1, Folin-Ciocalteu: distilled water) were mixed and left to stand for 5 minutes in room temperature. 1ml of 5% Na2CO3 was added to the reaction mixture and the reaction mixture was incubated for 1 hour in room temperature. After that the absorbance was read at 725nm, the values were converted to total phenolics and was expressed as (mg gallic acid equivalent/ ml yogurt).

2.7 DPPH Scavenging Activity

The measurement of the DPPH radical scavenging activity was determined by using the modified method of Shetty et al. (1995), by mixing 100µl of extraction sample with 2ml of dilute DPPH radicals. The mix was kept for 30 minutes in a dark room temperature. After that the absorbance was monitored at 517 nm. The reading was used to calculate the inhibition % of DPPH oxidation as (Apostolidis et al. 2007). Inhibition % = (A Control(517) – A Extract(517)/ A Control(517)x 100.

2.8 ABTS radical scavenging activity

The measurement of the ABTS radical scavenging activity was determined by using the modified method from the original method of Re et al. (1999). This assay made by mixing 7mM ABTS (2,2 '-azino-bis 3-ethylbenzothiazol-6-sulfonic acid) with 2.45 mmol L¯1 of potassium persulfate and puting it in the dark about 12-16h. The resultant solution was diluted with phosphate buffer saline (PBS) to obtain absorbance (0.700±2). After that the ABTS dilution was mixed with different concentrations of yogurt extraction samples, where the reaction was monitored over 6 min and the readings were measured at 734nm. The readings were converted to trolox equivalent antioxidant capacity (TEAC) and the results were expressed as mM trolox equivalent /g yogurt.

2.9 Microbiological analysis

Microbiological enumeration of yogurts was carried out at 1, 7, 14 and 21 days. The count of S.thermophilus was determined by aerobic incubated of culture M-17 Agar (7.2±0.2, Merck KGaA, Darmstadt, Germany) at 37°C. While the L.delbrueckii ssp. bulgaricus was estimated by anaerobic incubated of culture de Man Rogosa Sharpe Agar (MRS) (5.7 ±0.2, Merck KGaA, Darmstadt, Germany) at 37°C for 48h (Dave and Shah 1996). Testing for Yeast and moulds was made according to standard methods by using acidified Potato Dextrose Agar (PDA) (Merck KGaA, Darmstadt, Germany) (Marth 1978). Each medium freshly was prepared before each trial. Serial dilutions of the samples were made in sterile peptone water then transferred into plates.

2.10 Sensory evaluation

The common flavor, appearance, color, body and texture of all yogurt samples were evaluated sensorial by a trained panel of seven members using nine-point score system (1. Very bad, 2. Bad, 3. İmperfect, 4. Sufficient, 5. Mediocre, 6. Satisfactory, 7. Good, 8. Very good, 9. Excellent) (Obi et al. 2010).

2.11 Statistical analysis

Data analyzed by using Minitab 15 analysis software (State College PA, USA).

3. Results and Discussion

3.1 Chemical composition

The fat, protein, moisture, ash, total solid and solid-not-fat of RHMY were determined and compared to plain yogurt in the first day of storage period as shown in Table 1.

The RHM contributed in decreasing the value of fat and moisture, and increasing the protein, ash, total solid and solid-not-fat.

The increasing of protein can be related to the activity of micro-organisms that are presented in yogurt, which degraded fermentable sugars in the RHM into amino acids (Mahmood et al., 2008).

Table 1. The effect of rose hip marmalade on chemical compositions of yogurt

Rose hip marmalade concentration (%)		Fat (%)	Protein (%)	Ash (%)	Moisture (%)	SNF (%)	TS (%)
0 (plain yogurt)	Stir	3.19	3.70	0.85	85.72	11.09	14.28
	Set	3.20	3.69	0.83	85.93	10.87	14.07
5	Stir	3.02	3.72	0.89	82.94	14.04	17.06
	Set	3.05	3.74	1.01	82.78	14.17	17.22
10	Stir	2.50	3.75	0.90	81.99	15.51	18.01
	Set	2.70	3.77	1.03	81.75	15.55	18.25
15	Stir	1.90	3.85	1.07	81.44	16.66	18.56
	Set	2.2	3.80	1.05	80.79	17.01	19.21

The chemical composition of set and stirred yogurt after added the rose hip marmalade.

3.2 pH and titratable acidity

pH and titratable acidity were determined during 21 days of storage period as shown in Fig.1.1. RHMY showed significant decrease ($P<0.01$) in pH value during storage period. Similar results were reported by Öztürk and Öner (1999); Moon et al. (1993). The adding of different ratios of RHM affected the pH values significantly, where the 15% showed the lowest pH value and the 0% showed the highest value; similar results were reported by Öztürk and Öner (1999). The decreasing of pH value of yogurt can be influenced by the growth of both starter and non-starter lactic acid bacteria in pasteurized milk yogurt.

The significant ($P<0.01$) increasing acidity of RHMY with progress storage time, and with increasing marmalade added ratio (as shown in Fig.1.2.), can be related to the activity of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus subsp. thermophilus during the post-acidification period. Which produced lactic acid up to the availability of nutrients presented in yogurt. These results are compatible with Salji and Ismail (1983); Tarakçı and Küçüköner (2004); Çelik et al. (2006) results, which reported significant increasing in acidity along with storage.

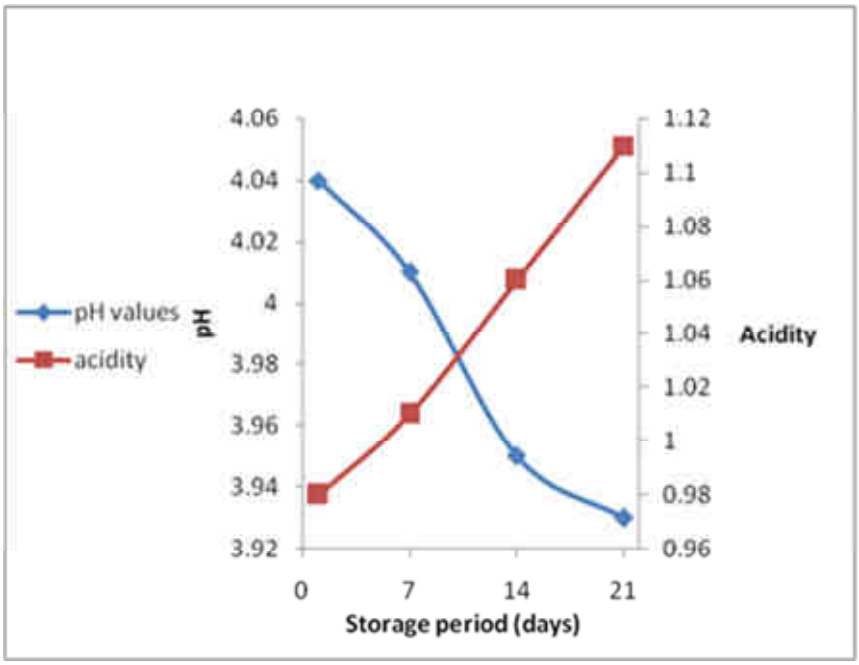

Figure 1.1. The pH and acidity changes of RHMY during storage period.

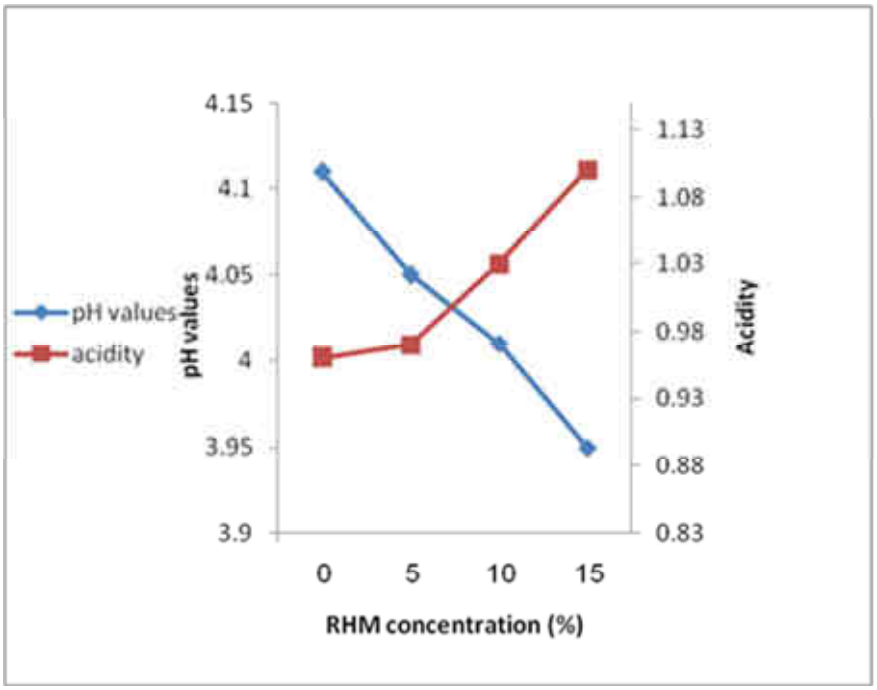

Figure 1.2. The pH and acidity changes of RHMY due to different marmalade concentrations.

3.3 Syneresis and water holding capacity

There were significant ($P<0.05$) differences in syneresis over 1, 7, 14, 21 days and different marmalade concentrations as shown in Fig.2.1&2.2. The values increased gradually up to 14th day then decreased gradually. During the progressing of storage time the increasing whey separation can be related to decreased water-holding capacity of protein (Calvo et al. 2001; Aryana et al. 2007; Öztürk and Öner 1999). Initially, the addition of RHM to yogurt causes a significant decreased ($P<0.05$) of syneresis values compared with plain yogurt. Yogurt formulated with 5% showed the highest syneresis, while the sample formulated with 15% showed the lowest syneresis, even lower than the plain sample. This could be related to the ability of absorbing water by the solid and fiber of RHM, which has higher water-holding ability (García-Pérez et al. 2005). The results were agreed with Tarakci and Kücüköner (2003) and Şengül et al. (2014).

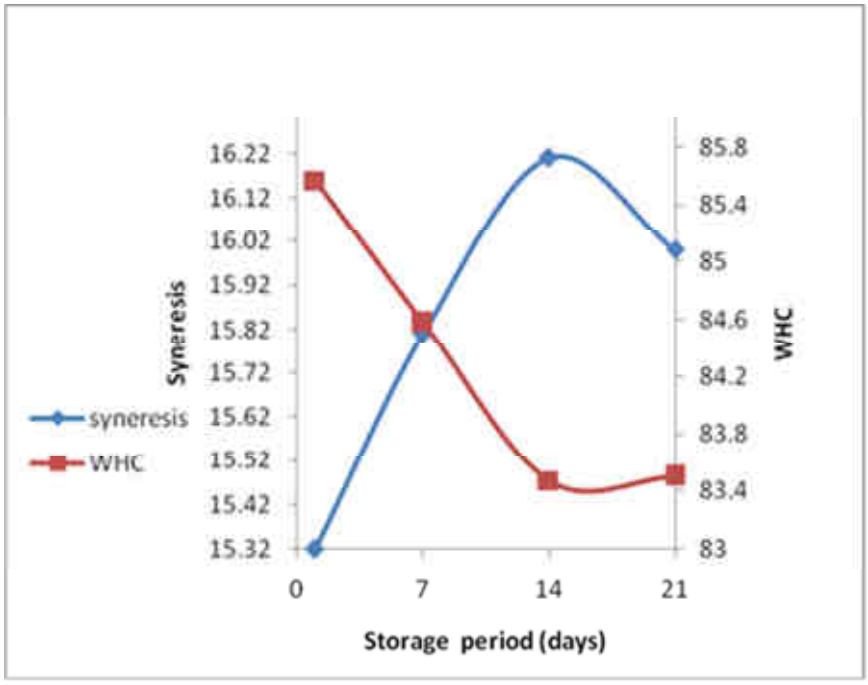

Figure 2.1. The whc and syneresis changes of RHMY during storage period.

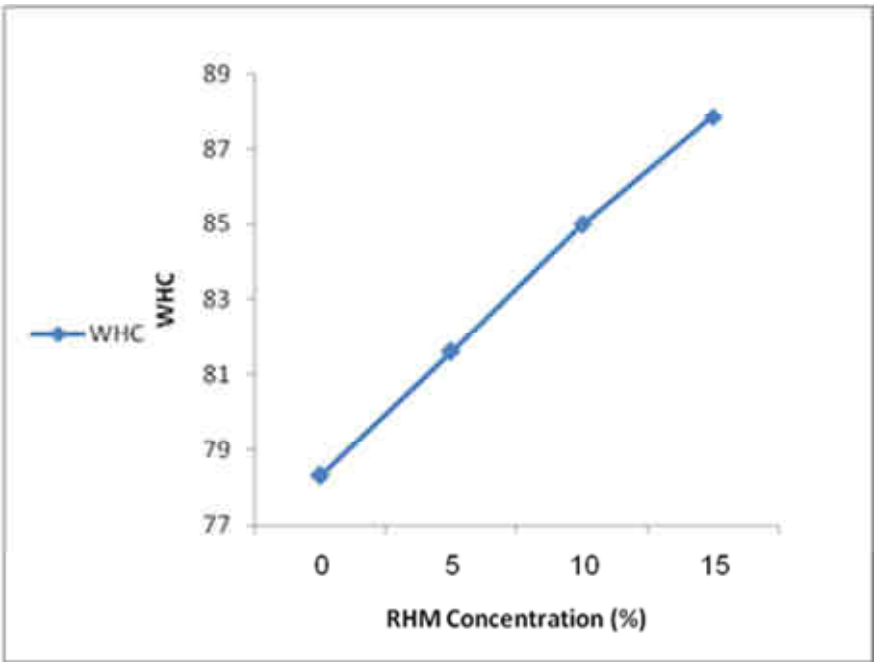

Figure 2.2. The whc change of RHMY due to different marmalade concentrations.

3.4 Total phenolic compounds

The phenolic content of RHMY was affected by the added ratio of marmalade and the advances of the storage period as shown in Fig.3.1&3.2. Where the phenolic compounds decreased significantly ($P<0.01$) in RHMY during 21 days of storage time. Similar results were reported by Karaaslan et al. (2011) when fortifying yogurt with grape and callus extracts; and Şengül et al. (2012) when added sour cherry to yogurt. Although the phenolic compounds of the fortified yogurt gradually decreased during the storage period, the phenolic compounds remained more than the plain yogurt.

The phenolic compounds increased significantly ($P<0.05$) by increasing the amount of added marmalade, where the highest score were observed in the addition of 15% marmalade compared with plain sample. Similar results were reported by Zainoldin and Baba (2009) when fortifying yogurt with dragon fruit, and by Şengül et al. (2012) when fortifying yogurt with sour cherry pulp.

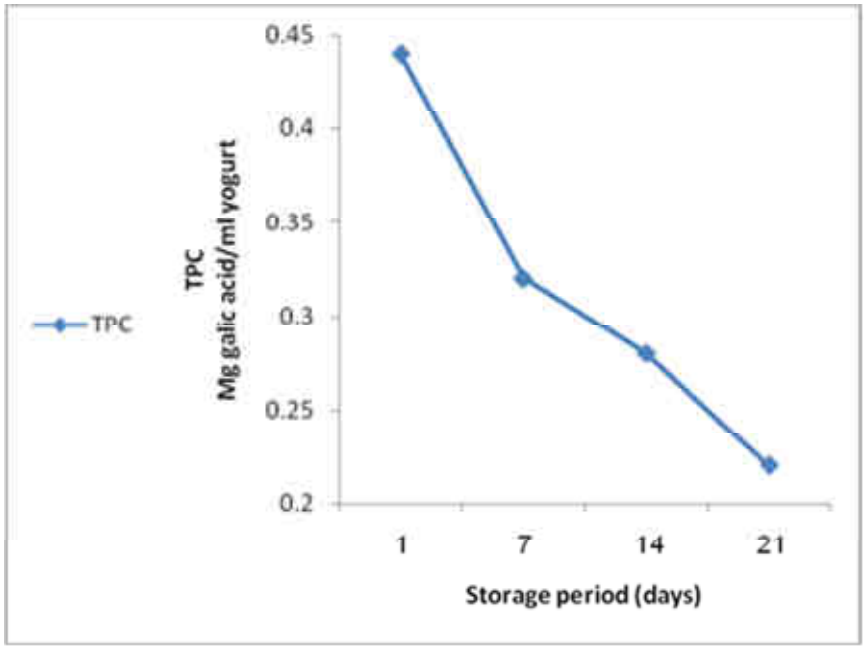

Figure 3.1. TPC change of RHMY during storage period.

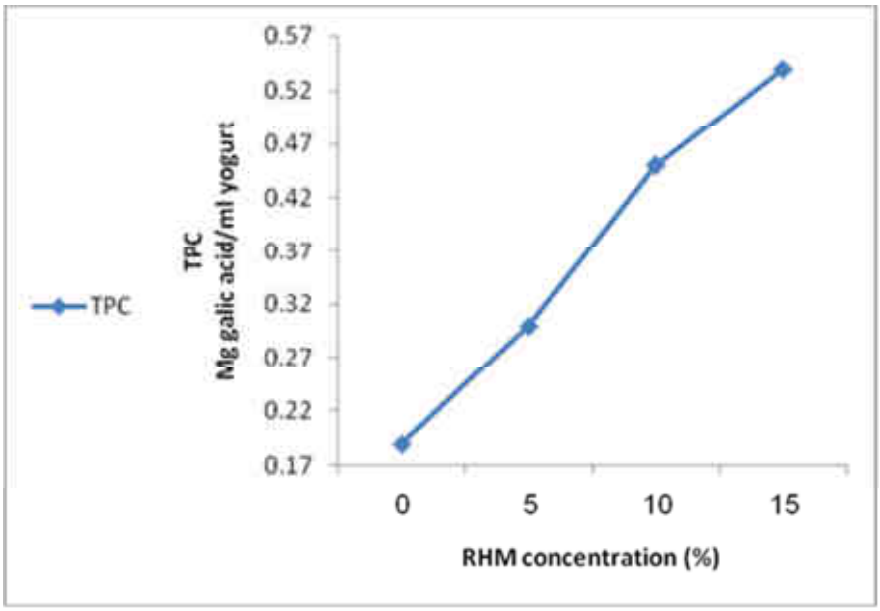

Figure 3.2. TPC change of RHMY due to different marmalade concentrations.

3.5 DPPH scavenging activity

The scavenging activities of RHMY to DPPH radicals decreased significantly (P<0.1) with storage time, that means the antiradical capacities of RHMY vanished by time as shown in Fig.4.1. This vanishing can be related to the increased degradation of phenolic compounds with antioxidant activities (Yildiz and Eyduran 2009) or increased milk protein-polyphenol interaction (Yuksel et al. 2010). Otherwise the scavenging activities of DPPH radicals by RHMY increased significantly (P<0.1) with the increasing of the added ratio of RHM comparing with plain yogurt as shown in Fig.4.2. This can be related to the high phenolic compounds and valuable sources of vitamin C, flavonoids, tannins, carotenoids (β-carotene, licopene, and isomeres of rubixanthin) and organic acids of RHM (Pârvu, 2000; Demir and Ozcan, 2001; Tiţă, 2003; Stănescu et al., 2004; Arsenescu, 2008; Orhan et al., 2009).

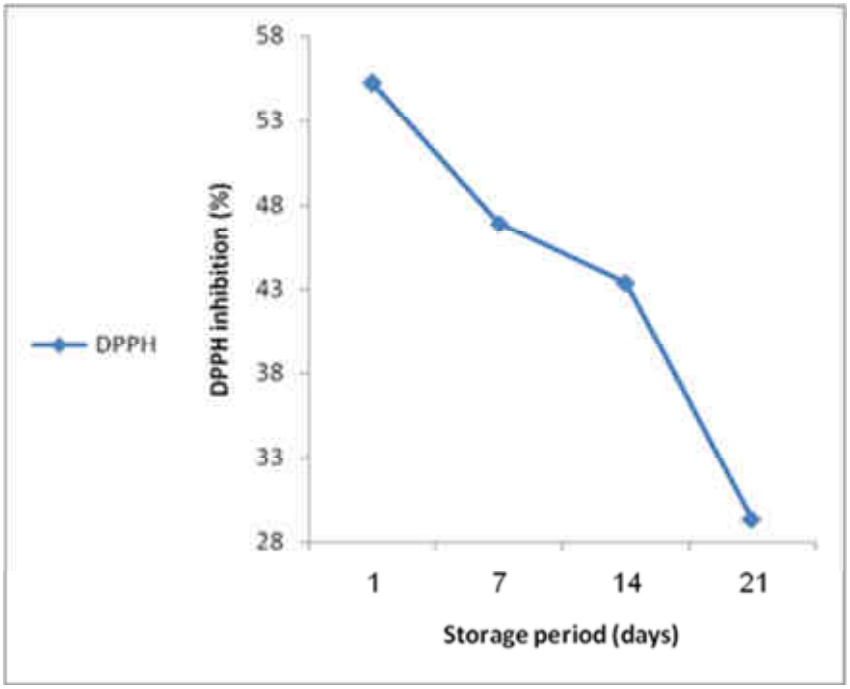

Figure 4.1. DPPH inhibition changes of RHMY during storage period.

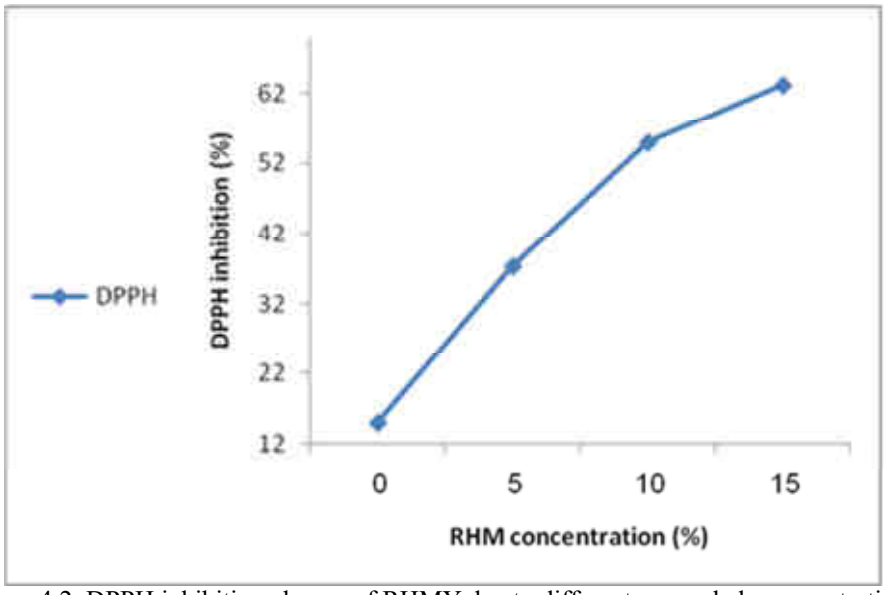

Figure 4.2. DPPH inhibition change of RHMY due to different marmalade concentrations.

3.6 ABTS radical scavenging activity

According to ABTS assay the ABTS+ scavenging of RHMY decreased significantly ($P<0.05$) by the progress of storage period as shown in Fig.5.1&5.2.

The decreasing in ABTS+ scavenging activity of RHMY during storage period agreed with results obtained by Oliveira et al. (2015) when observing decrease in ABTS+ score during 21 days of storage in strawberry mixed yogurt.

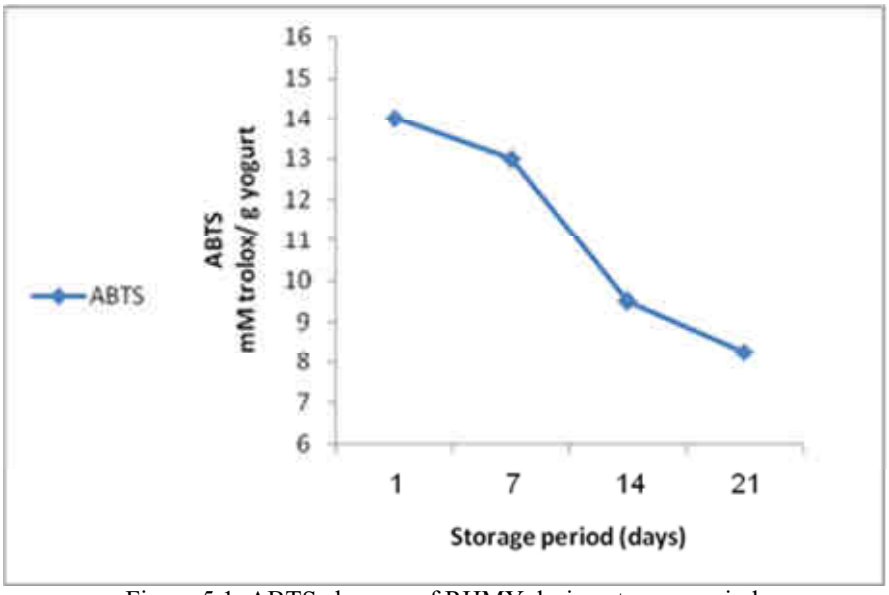

Figure 5.1. ABTS changes of RHMY during storage period.

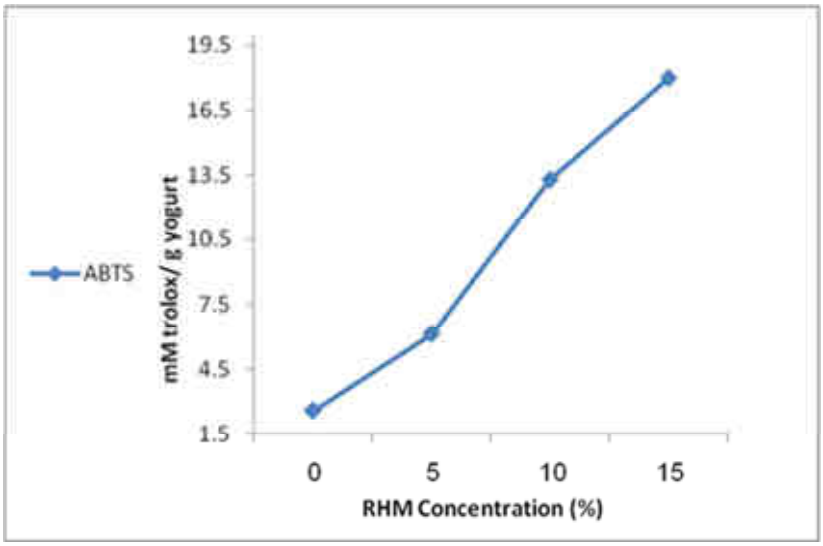

Figure 5.2. The ABTS changes of RHMY due to different marmalade concentrations.

3.7 The microbiological enumeration

The effects of storage period and the different concentrations of marmalade on the microbiological characteristics of RHMY are shown in Table 2&3.

Table 2. The effect of storage period on the microbiological characteristics of RHMY

Storage period (days)		Total bacteria	Lactobacillus Log CFU/g	Streptococcus
1st	Stir	8.86±0.00	7.91 ± 0.09^{A}	7.59 ± 0.25^{E}
	Set	8.47±0.90	7.72 ± 0.08^{B}	7.96 ± 0.14^{D}
7th	Stir	8.51±0.02	7.63 ± 0.10^{C}	8.09 ± 0.20^{C}
	Set	8.19±0.40	7.60 ± 0.01^{C}	8.13 ± 0.08^{B}
14th	Stir	8.36±0.08	7.98 ± 0.07^{A}	8.14 ± 0.01^{B}
	Set	8.02±0.01	6.89 ± 0.07^{D}	8.16 ± 0.05^{B}
21st	Stir	8.22±0.20	6.87 ± 0.02^{D}	8.25 ± 0.03^{A}
	Set	7.93±0.15	6.77 ± 0.21^{D}	8.29 ± 0.13^{A}

The (A,B,C,D) in the data have statistical significant difference ($p<0.01$) between storage period of yogurt.

Table 3. The effect of different marmalade concentrations on the microbiological characteristics of RHMY

Rose hip marmalade concentration (%)		Total bacteria	Lactobacillus Log CFU/g	Streptococcus
0 (plain yogurt)	Stir	8.33 ± 1.02^{E}	8.27 ± 0.82^{D}	7.58 ± 1.52^{F}
	Set	8.22 ± 0.00^{G}	8.32 ± 0.24^{C}	7.69 ± 1.02^{F}
5	Stir	8.32 ± 1.00^{E}	8.42 ± 0.12^{B}	8.30 ± 0.77^{C}
	Set	8.28 ± 0.05^{F}	8.51 ± 0.05^{A}	7.82 ± 0.09^{E}
10	Stir	8.38 ± 0.42^{C}	8.38 ± 0.62^{C}	8.39 ± 0.85^{B}
	Set	8.34 ± 0.03^{D}	8.22 ± 0.01^{C}	7.89 ± 0.42^{D}
15	Stir	8.46 ± 0.22^{A}	8.30 ± 0.42^{D}	8.43 ± 0.82^{A}
	Set	8.43 ± 0.15^{B}	7.58 ± 0.00^{E}	7.91 ± 0.32^{D}

The (A,B,C,D) in the data have statistical significant difference ($p<0.01$) between different marmalade concentrations of yogurt.

3.8 sensory evaluation

The sensory evaluation affected by different added concentrations of RHM to yogurt as well as the progress of storage period. Fortifying the yogurt with 10% RHM shows the highest acceptance comparing with plane and other treated yogurt. While fortifying the yogurt with 5% and 15% show less acceptance comparing with plain yogurt. According to storage period the RHMY shows less acceptance with the progress in storage period. Where the shelf-lives at 4°C±2 of RHMY should be 14 days. The acceptability of yogurt by panellists at 21st day was not evaluated because the product's appearance was not quite acceptable.

4. Conclusion

Initially, the RHM contributes to enhancing the antioxidant activity and water holding ability of yogurt due to its high concentration of phenolic compounds, vitamins, fiber and pectin. The acidity of RHM contributes to decline overall acceptableness of RHMY during last stage of storage period.

The consumption of RHMY is highly advisable within 7 days after its preparation to benefit from the high live bacterial contents and high antioxidant activities.

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