Effect of Heat Treatments on Antioxidant Activity in Sucrose-Milk Protein Model Systems

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ABSTRACT:

An investigation was carried out to estimate the antioxidant activity in simulated milk beverage model systems at different heat treatments equivalent to industrial processing of dairy beverages. Model systems of sucrose and milk proteins (whey protein and casein) were given different heat treatments to generate Maillard reaction products. These MRPs were considered to elevate the antioxidant activity in dairy beverages. Percentage inhibition of DPPH as a measure of antioxidant activity was determined at different time intervals. The study showed that there was a significant difference in the free radical scavenging activity of samples at different time intervals at a given temperature.

KEYWORDS: *Antioxidant, Free radical scavenging activity, HMF, Maillard reaction, Proteins, Sucrose,*

I. INTRODUCTION

Consumption of milk confers a number of nutritional benefit [1]. Besides the valuable macro and micro-nutrients, milk also contains antioxidant factors. These are due to by naturally occurring vitamins (i.e. E and C), beta carotene and enzymatic systems mainly superoxide dismutase, catalase and glutathione peroxidase [2]. Furthermore, it has been reported that milk antioxidant activity increases as a consequence of thermal treatments, due to protein unfolding and exposure of thiol groups, potentially acting as hydrogen donors [3,4]. Almost all dairy products are manufactured from heated milk because of specific reasons involving microbial stability and safety, shelf-life extension, or technological aspects related to product functionality or quality [5]. Depending on the intensity of the thermal treatment applied, pro-oxidant or antioxidant molecules are expected to be produced. The Maillard reaction has been used to produce foods that look and taste attractive for thousands of years, for as long as food has been cooked [6]. Both caramelization and the Maillard reaction are responsible for the development of attractive colours and flavours in heat-processed foods.The modern food industry relies on the application of Maillard reaction products to produce many foods like coffee, bakery and dairy products that possess the color and flavor demanded by the consumers. Some indigenous milk products like *khoa,* flavored milk, *burfi, kalakand* also undergo such reactions during prolonged heating and impart characteristic color and flavor which is liked by the people of Indian continent [7].The Maillard reaction is a complex network of reactions that occur during processing and storage of several foodstuffs. Several methods have been used for determining the extent to which it has progressed, such as the 2-thiobarbituric acid (TBA) method, periodate oxidation, borohydride reduction, and furosine and carboxy methyllysine determination [8]. Many studies have appeared on the application of HMF as an index of heat treatments in milk products and other foodstuffs. 5-(Hydroxymethyl)-2- furfuraldehyde (HMF) is formed upon heat treatment of milk and milk resembling systems by the Maillard reaction, via its Amadori product, and also by isomerization and subsequent degradation of sugars. Traditionally, the HMF content has been used as an indicator of both degradation routes.

Maillard reaction products generated from sugar-protein model in food materials during processing and storage have strong antioxidant activity [9]. The antioxidant activity of MRPs was first reported [10] and has been extensively investigated thereafter [11]. The higher interaction between lactose and proteins in milk having higher pH value could lead to more Maillard Reaction Products (MRP) as well as more polymerisation of protein [12]. When foods are heat processed, the sugars and lipids react with the proteins they contain via the Maillard and related reactions to form a wide range of products. As a result, the sensory, safety, nutritional and health-promoting attributes of the foods are enhanced [13]. Some fractions were reported to have strong antioxidant properties comparable to those of commonly used food antioxidants [14]. The action mechanisms are supposed to involve radical chain-breaking activity [15], metal-chelating ability [16], active oxygen species scavenging and hydrogen peroxide destroying ability [17, 18]. The Maillard reaction occurs in three stages (early, intermediate and final stage), and is dependent upon factors such as reactants type and concentration, temperature, time, pH and water activity [19].

Upon heating foods at high temperature, 5-hydroxy- methyl-2-furfuraldehyde (HMF) is naturally generated by two possible pathways: (1) caramelization, where the reducing carbohydrates, including maltose and maltotriose [20], directly undergo 1-2 enolization, dehydration and cyclization reactions; and (2) the Maillard reaction, where the Amadori product, formed by reaction with the amino group of free amino acids or proteins, undergoes enolization and subsequent dehydration of the sugar moiety while releasing the amino acid intact [21]. Current knowledge is not sufficient to identify the technological conditions which either promote or inhibit the formation of antioxidative components in milk products. For these reasons, it would be valuable to determine the processing conditions that improve antioxidant potential and minimise oxidative reactions responsible for a decline of milk quality attributes. Research considering the antioxidant activity of MRPs has been performed mostly with sugar-amino acid models, relatively less is known about the antioxidant activity potential of sucrose-milk protein models representing the effect of heat treatment on sweetened dairy beverages. Since most dairy beverages contain native milk proteins (whey proteins and casein) and sucrose (as sweetening agent), the purpose of this study was to determine the effect of heat treatments below and above 100 °C on the antioxidant activity of milk beverages. This knowledge could provide information not only on the overall health protecting potential of milk products but also on the stability of complex foods containing milk.

II. MATERIALS AND METHODS:

Milk was procured from College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU)**. S**ucrose was purchased from local market of Ludhiana, Punjab. Whey protein concentrate (70 %) was supplied by Mahaan proteins, New Delhi, soluble casein (99.9 %) was made available by SD fine chemicals. DPPH was obtained from Sigma Aldrich, USA. All the chemicals used were AR grade.

2.1. Milk samples

Thirteen samples were prepared using different combinations of Whey protein concentrate (0.5 to 1.0%), Casein (0.5 to 1.0%), and sucrose (6%) in 100 mM Phosphate Buffer (pH 7.0). Skimmed milk (0.5 % milk fat) with and without sugar were taken as control samples. All the samples were subjected to different heat treatments equivalent to pasteurization and sterilization of a dairy beverage to generate Maillard Reaction Products.

Variables	**Temperature**	**Time**
	63 °C	30, 40, 50 min
	73 °C	15, 30, 45 sec
	83 °C	15, 20, 25 sec
	110 °C	25, 30, 35 min
	116 °C	20, 25, 30 min
	121 °C	15, 20, 25 min

Model solutions were heated in stoppered test tubes in a water bath at temperature from 63 to 73°C. For temperature above 100°C, the samples were autoclaved at different pressures to attain required temperature (5 psi for 110°C, 10 psi for 116°C and 15 psi for 121°C). After pre-determined heat treatments, the samples were immersed in ice bath for rapid cooling. Thereafter, samples were stored at 4°C and analyzed within 3 h. All analyses were performed in triplicate.

Chemical analysis

Antioxidant Activity estimation

Antioxidant activity was estimated using standard procedure of DPPH (2, 2-diphenyl1-picrylhydrazyl) assay [22]. Six ml of DPPH solution (0.2mM in 80% methanol) was mixed with 2 ml of each sample. The samples were incubated for 30 min. in dark. The absorbance (A) was measured at 518 nm in Spectronic-20 (Bausch and Lomb, USA) spectrophotometer. The percentage of the radical scavenging activity was calculated as percentage inhibition of DPPH radicals using the following equation:

% inhibition of DPPH = [A control – A sample] / A control × 100

Two ml Methanol (80%) plus 6 ml distilled water was used as a blank. In control, DPPH solution replaced distilled water.

Statistical analysis

Factorial CRD with multiple replications was carried out and difference between means was obtained using CPCS-1 software developed by the Department of Mathematics and Statistics, PAU, Ludhiana, India. All the statistical procedures were performed at a significance level of 95%.

III. RESULTS AND DISCUSSION

Effect of heat treatment (below 100 °C) on Antioxidant activity

There was a significant increase in the antioxidant activities in samples as the heating time increased at a given temperature (Table 1). The antioxidant activity of the control sample was found lowest while sample nine showed antioxidant activity during heating. This may be due to the higher amounts of casein as well as whey protein present in sample nine which react with sucrose to generate augmented amounts of MRPs. Whey proteins and casein have more amount of lysine amino acid. This lysine is highly reactive with sucrose to generate MRPs readily [23].Heat treatment below 100°C showed that most of the samples were significantly different for antioxidant activity values at the initial stage. It indicates that the amount and nature of substrate has a marked effect on the generation of MRPs, which may affect the antioxidant activity. Similarly, at the later stages of heating (above 100°C) there was a significant difference observed between all the samples in terms of antioxidant activities. It was noted that natural milk system (skimmed milk) had antioxidant activity of 24.13 which increased to 32.49 in presence of 6 percent sucrose. This indicates that presence of sucrose in milk beverage has a marked effect on MRP generation. This may be due to the fact that antioxidants activity in biological systems is dependent on a multitude of factors, including the colloidal properties of the substrates, the conditions and stages of oxidation, temperature, pH, time of heating and the localization of antioxidants in different phases. Intervals can be accounted for the MRPs whose antiradical properties are well documented [24]. The findings are in agreement with the previous data from Taylor and Richardson (1980) which states that heat treatment of milk is associated with the increase in antioxidant activity.

Earlier found that the free radical scavenging activities of different model systems containing glucose or lactose sugar with lysine, alanine or glycine amino acids increased drastically during heating [25]. Such an increase was detected over different time frames depending on the heating temperature. However, the metal ion binding of MRPs could represent a mechanism for antioxidant activity more than direct free radical scavenging activity [26]. Calligaris et al. (2004) stated that food products heated at 90 °C or above even for seconds may show a significant change in antioxidant activity related to MRP generation in the system. The increase in free radical scavenging activity of MRPs increased from 0 to 70% as the heating time increased from 0 to 10 h at constant temperature [28].

Effect of heat treatment (above 100 °C) on Antioxidant activity

Heat treatments above 110°C led to the generation of brown colored samples. This color development was mainly due to the formation of chromophores, which have been widely studied in different model systems [29, 30, 31]. The development of Maillard reaction was clearly indicated by milk browning, which promptly occurred when heat treatment was performed at 120°C and slowly developed after 1.5- and 2-h heating at 80°C and 90°C, respectively. Higher the temperature yielded more MRPs that is why the antioxidant activities at elevated temperatures had higher values. Maillard reactions occur slowly at 35 °C, but are accelerated at a temperature 55°C or greater [32]. The brown color generated from sucrose-protein model systems are mainly due to the formation of protein oligomers that are mediated by chromophoric sub structures derived from carbohydrates [33]. Moreover recently attempted to correlate the biological and chemical effects of MRPs with the browning rates [34].

IV. CONCLUSION

The functionality of several heat-induced parameters in relation to antioxidant activity as a result of Maillard Reaction Products (MRPs) generation has been studied on different sucrose-protein model systems. The extent of generation of MRPs during the heat treatment may be evaluated using antioxidant activity as an index. Antioxidant activity increased significantly with increase in heating time at a given temperature. The simultaneous application of several heat-induced parameters may improve the classification of industrial processes of milk and dairy products, yielding a useful tool for optimization of processing conditions for better functionality and stability of the prepared dairy products.

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Table 1 Effect of heat treatment (below 100 °C) on Antioxidant activity

Sr.No	Treatments (%)			63 °C				73 °C				83 °C			
	Whey Protein	Casein	Sugar	30 min	40 min	50 min	Mean	15 sec	30 sec	45 sec	Mean	15 sec	20 sec	25 sec	Mean
1	0	0	0	$0.01^{h,1}$	$0.01^{i,2}$	$0.01^{i,3}$	0.01	$0.01^{i,1}$	$0.01^{i,2}$	$0.01^{i,3}$	0.01	$0.01^{m,1}$	$0.01^{i,2}$	$0.01^{k,3}$	0.01
2	0	0.5	6	$19.37^{e,1}$	$32.72^{e,f,2}$	$42.30^{e,3}$	31.46	$28.38^{f,1}$	$42.38^{b,2}$	$59.32^{f,3}$	43.36	$32.57^{j,1}$	$36.28^{j,2}$	$39.38^{i,3}$	36.08
3	0	1	6	$24.67^{c,d,1}$	$36.42^{d,2}$	$45.59^{d,3}$	35.56	$34.66^{c,1}$	$54.65^{f,2}$	$61.34^{e,3}$	50.22	$36.26^{h,1}$	$42.33^{g,2}$	$48.46^{f,3}$	42.35
4	0.5	0	6	$11.45^{g,1}$	$28.30^{g,2}$	$39.37^{f,3}$	26.37	$33.49^{f,1}$	$54.55^{f,2}$	$57.24^{g,3}$	48.42	$33.20^{i,1}$	$38.25^{i,2}$	$43.52^{h,3}$	38.32
5	0.5	0.5	6	$23.50^{d,1}$	$44.61^{c,2}$	$48.29^{c,3}$	38.80	$40.30^{g,1}$	$64.73^{e,2}$	$67.36^{d,3}$	57.46	$42.28^{g,1}$	$49.43^{e,2}$	$51.42^{e,3}$	47.71
6	0.5	1	6	$26.57^{b,c,1}$	$58.42^{a,2}$	$53.28^{b,3}$	46.09	$46.39^{e,1}$	$66.75^{d,2}$	$69.48^{c,3}$	60.88	$48.15^{c,1}$	$54.47^{d,2}$	$62.25^{c,3}$	54.96
7	1	0	6	$16.46^{f,1}$	$30.36^{f,g,2}$	$46.28^{c,d,3}$	31.03	$52.60^{d,1}$	$68.44^{c,2}$	$69.48^{c,3}$	63.51	$45.18^{e,1}$	$49.31^{e,2}$	$59.31^{d,3}$	51.27
8	1	0.5	6	$27.28^{a,b,1}$	$48.25^{b,2}$	$52.39^{b,3}$	42.64	$57.34^{c,1}$	$72.43^{b,2}$	$74.36^{b,3}$	68.05	$46.14^{d,1}$	$57.57^{b,2}$	$63.39^{b,3}$	55.70
9	1	1	6	$29.51^{a,1}$	$59.31^{a,2}$	$59.30^{a,3}$	49.37	$58.62^{b,1}$	$75.47^{a,2}$	$79.35^{a,3}$	71.15	$52.58^{a,1}$	$65.49^{a,2}$	$75.50^{a,3}$	64.52
10	0.738	3.012	6	$11.85^{g,1}$	$20.48^{i,2}$	$36.58^{e,3}$	22.97	$22.42^{a,1}$	$13.46^{k,2}$	$16.39^{k,3}$	17.42	$13.46^{l,1}$	$18.43^{k,2}$	$25.44^{j,3}$	19.11
11	Skimmed milk		-	$17.23^{e,f,1}$	$25.63^{h,2}$	$29.54^{h,3}$	24.13	$26.64^{k,1}$	$32.62^{j,2}$	$41.47^{j,3}$	33.58	$31.52^{k,1}$	$40.62^{h,2}$	$46.34^{g,3}$	39.49
12	Skimmed milk		6	$24.64^{c,d,1}$	$33.40^{e,2}$	$39.44^{f,3}$	32.49	$32.67^{j,1}$	$38.53^{i,2}$	$44.25^{i,3}$	38.48	$40.39^{g,1}$	$45.78^{f,2}$	$51.57^{e,3}$	45.91
13	0.738	3.012	-	$27.58^{a,b,1}$	$37.40^{d,2}$	$42.59^{e,3}$	35.86	$38.13^{h,1}$	$45.46^{g,2}$	$49.31^{h,3}$	44.22	$51.22^{b,1}$	$55.58^{c,2}$	$59.46^{d,3}$	55.42
Mean				20.02	35.02	41.15		36.28	48.42	53.03		36.38	42.58	48.16	

*significant at p≤0

Means with different superscripts (a, b…m) differ significantly (p≤0.05) in a column
Means with different superscripts (1, 2, 3) differ significantly (p≤0.05) in a row

Source	Df	MSS		
		110 °C	116 °C	121 °C
A(Time)	2	1972.2860*	1517.1750*	1655.2970*
B (Samples)	12	3028.5660*	2951.7350*	2876.9730*
AXB	24	34.217610*	45.296220*	63.484540*
ERROR	78	.25120190	.27909650	28.730120

*significant at p≤0.05

Table 2 Effect of heat treatment (above 100 °C) on Antioxidant activity

Sr.No	Treatments (%)			110 °C				116 °C				121 °C			
	Whey Protein	Casein	Sugar	25 min	30 min	35 min	Mean	20 min	25 min	30 min	Mean	15 min	20 min	25 min	Mean
1	0	0	0	$0.01^{b,1}$	$0.01^{m,2}$	$0.01^{m,3}$	0.01	$0.01^{l,1}$	$0.01^{m,2}$	$0.01^{l,3}$	0.01	$0.01^{c,1}$	$0.01^{c,2}$	$0.01^{f,3}$	0.01
2	0	0.5	6	$14.47^{i,1}$	$18.2^{i,2}$	$129.04^{b,3}$	20.67	$24.44^{b,1}$	$31.45^{b,2}$	$37.91^{i,3}$	31.27	$27.35^{f,1}$	$33.15^{f,2}$	$41.45^{e,3}$	33.98
3	0	1	6	$24.44^{b,1}$	$30.20^{f,2}$	$43.09^{b,3}$	32.57	$27.66^{i,1}$	$35.53^{i,2}$	$45.71^{i,3}$	63.30	$33.16^{e,1}$	$37.46^{e,f,2}$	$46.46^{e,3}$	39.03
4	0.5	0	6	$37.20^{e,1}$	$50.28^{e,2}$	$57.41^{e,3}$	48.30	$51.62^{e,1}$	$59.43^{e,2}$	$62.23^{e,3}$	57.76	$54.47^{a,b,1}$	$61.75^{b,2}$	$68.13^{b,3}$	61.45
5	0.5	0.5	6	$25.49^{g,1}$	$32.57^{h,2}$	$47.70^{f,3}$	35.25	$29.39^{h,1}$	$36.43^{h,2}$	$53.47^{f,3}$	39.76	$39.28^{d,1}$	$41.75^{d,e,2}$	$55.34^{e,3}$	45.46
6	0.5	1	6	$55.47^{b,1}$	$61.50^{b,2}$	$65.80^{b,3}$	60.92	$57.45^{b,1}$	$64.48^{b,2}$	$68.11^{b,3}$	63.35	$58.24^{a,1}$	$66.46^{a,2}$	$69.44^{b,3}$	64.71
7	1	0	6	$41.45^{d,1}$	$45.42^{g,2}$	$53.70^{e,3}$	46.86	$49.35^{g,1}$	$51.52^{g,2}$	$53.35^{f,3}$	51.41	$51.15^{b,1}$	$61.70^{b,2}$	$65.41^{b,3}$	59.42
8	1	0.5	6	$53.86^{a,1}$	$63.28^{b,2}$	$64.44^{a,3}$	60.53	$55.61^{a,1}$	$66.45^{a,2}$	$67.33^{a,3}$	63.12	$55.69^{a,b,1}$	$68.89^{a,2}$	$69.57^{b,3}$	64.72
9	1	1	6	$56.48^{a,1}$	$65.22^{a,2}$	$68.87^{a,3}$	63.53	$61.35^{a,1}$	$65.50^{b,2}$	$75.55^{a,3}$	67.47	$44.56^{d,1}$	$68.46^{a,2}$	$79.11^{a,3}$	64.04
10	0.738	3.012	6	$14.32^{i,1}$	$22.73^{k,2}$	$26.53^{i,3}$	21.19	$27.32^{i,1}$	$28.48^{i,2}$	$34.42^{k,3}$	30.07	$33.21^{e,1}$	$34.51^{f,2}$	$43.17^{d,e,3}$	36.96
11	Skimmed milk		-	$19.50^{j,1}$	$28.35^{j,2}$	$38.55^{j,3}$	28.80	$26.83^{j,1}$	$39.17^{k,2}$	$49.26^{g,3}$	38.42	$41.64^{c,d,1}$	$44.41^{c,d,2}$	$55.24^{e,3}$	47.09
12	Skimmed milk		6	$26.91^{g,1}$	$36.49^{i,2}$	$39.43^{i,3}$	34.28	$39.58^{f,1}$	$48.35^{f,2}$	$47.35^{h,3}$	45.15	$45.55^{c,1}$	$47.35^{c,2}$	$47.59^{d,3}$	46.83
13	0.738	3.012	-	$25.39^{g,1}$	$35.44^{g,2}$	$45.31^{g,3}$	35.38	$36.55^{g,1}$	$42.78^{g,2}$	$54.45^{e,3}$	44.60	$44.83^{c,1}$	$48.63^{c,2}$	$57.59^{e,3}$	50.35

Means with different superscripts (a, b…m) differ significantly (p≤0.05) in a column
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*significant at p≤0.05