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FOOD TECHNOLOGY

Effect of Industrial Processing on HMF Generation in Sucrose-Milk Protein Model Systems

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ABSTRACT

An investigation was carried out to estimate the HMF concentration at different heat treatments equivalent to industrial processing of milk and dairy beverages. Model systems of sucrose and milk proteins (whey protein and casein) were given heat treatments to generate Maillard reaction products. HMF concentration (mg/100g) was determined at different time intervals. One of the methods is 5-(Hydroxymethyl)-2-furfuraldehyde (HMF) estimation. This could provide information not only on the overall health protecting potential of milk products but also on the stability of complex foods containing milk. In this research, simulated milk beverages were prepared to get an idea of HMF generation during industrial processing of dairy products. The results revealed that temperature and duration both have a significant effect on generation of HMF which directly correlation with generation of antioxidative maillard reaction products.

Highlights

- The study showed that there was a significant difference in the HMF concentration of samples at different heat treatments. During the thermal treatments given to milk and milk products during industrial processing, maillard reactions products (MRPs) are generated as a result of reaction between sugars and amino acids. These MRPs impart characteristic color and flavor to the product.

Keywords: Maillard reaction, proteins, sucrose, HMF

Maillard reactions lead to changes in food color, organoleptic properties, protein functionality, and protein digestibility. Numerous different strategies for controlling Maillard reactions in foods have been attempted during the past decades. Maillard reactions are initiated by a condensation of amino groups on protein, peptides, and amino acids with carbonyl groups on reducing sugars, resulting in Schiff base formation and rearrangement to Amadori or Heyns products Maillard reaction intermediates include furfural, 5-(hydroxymethyl) furfural (HMF), reductones, and acrylamide. Eventually, large polymeric compounds, melanoidins, are formed, which causes browning (Hellwig and Henle 2014). Altogether, these lead to major compositional, structural, and functional changes to food components, including proteins, amino acids, and sugars (Pischetsrieder, and Henle 2012), and have potentially significant implications for food color, taste, protein functionality, and digestibility of foods (Toda *et al.* 2014). Maillard reaction products generated from sugar-protein model in food materials during processing and storage have strong antioxidant activity (Rao *et al.* 2011). The antioxidant activity of MRPs was first reported by Franzke and Iwainsky (1954) and has been extensively investigated thereafter (Benjakul *et al.* 2005). 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 proteins (Gothwal and Bhavadasan 1992). 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 (Ames, 2009). Some fractions were reported to have strong antioxidant properties comparable to those of commonly used food antioxidants (Lingnert and Hall 1986). The action mechanisms are supposed to involve radical chain-breaking activity (Morales and Babbel 2002), metal-chelating ability (Bersuder *et al.* 2001), active oxygen species scavenging (Wagner *et al.* 2002) and hydrogen peroxide destroying ability (Wijewickreme *et al.* 1999, Giroux *et al.* 2008). 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 (Hwang *et al.* 2011).

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 (Kroh 1994), 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.

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 equivalent to pasteurization and sterilization of milk beverages on antioxidant activity and HMF generation. 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.

MATERIALS AND METHODS

Milk was procured from College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU). Sucrose was purchased from local market of Ludhiana, Punjab. Whey protein concentrate (70 %) was supplied by Mahan 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.

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. HMF concentration (mg/100g) was obtained as an index of MRPs generation.

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, 25, 30 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.

Table 1: HMF concentration at temperatures below 100°C

Sl. 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	0.01e	0.01^{g}	0.01^{h}	0.01^{l}	0.01^{f}	0.01^{g}	0.01^{g}	0.01^{i}	0.01^{h}	0.01^{h}	0.01^{f}	0.01^{h}
2	0	0.5	6	115.99^{d}	266.82^{e}	320.07^{f}	234.34^{b}	191.67^{e}	283.54^{e}	617.51^{b}	364.24^{h}	230.65^{f}	320.29^{f}	490.94^{c}	347.29^{e}
3	0	1	6	150.33^{c,d}	500.66^{c}	517.78^{d}	389.59^{f}	516.65^{a,b}	598.72^{b}	634.87^{b}	583.41^{d}	485.23^{d,e}	493.94^{d}	530.91^{c}	503.36^{d}
4	0.5	0	6	141.41^{c,d}	350.79^{d}	400.28^{e}	297.49^{g}	367.07^{d}	398.85^{a,b}	433.21^{d}	399.71^{g}	266.36^{f}	421.17^{e}	462.18^{c}	383.23^{e}
5	0.5	0.5	6	166.74^{c}	523.35^{c}	574.69^{d}	421.59^{e}	498.17^{b,c}	467.65^{c}	683.98^{a,b}	549.93^{e}	541.72^{c,d}	597.69^{c}	534.50^{c}	557.97^{c}
6	0.5	1	6	217.12^{a,b}	733.19^{b}	837.19^{b}	595.83^{b}	562.37^{a}	687.10^{a}	734.55^{a}	661.34^{b}	637.14^{a,b}	680.32^{a,c}	731.64^{a,b}	683.03^{a}
7	1	0	6	150.89^{c,d}	484.05^{c}	667.56^{c}	434.17^{d}	434.52^{c}	447.49^{c,d}	533.10^{c}	471.71^{f}	450.32^{e}	521.21^{d}	534.57^{c}	502.06^{d}
8	1	0.5	6	175.05^{b,c,d}	817.60^{a}	734.41^{c}	575.69^{c}	547.08^{a,b}	563.86^{b}	690.46^{a,b}	600.47^{c}	581.26^{b,c}	632.04^{b,c}	674.45^{b}	629.25^{b}
9	1	1	6	251.01^{a}	884.58^{a}	935.17a	690.25^{a}	566.70^{a}	696.64^{a}	756.22^{a}	673.19^{a}	655.39^{a}	735.71^{a}	793.85^{a}	728.32^{a}
10	0.738	3.012	6	135.77^{c,d}	142.96^{f}	154.31^{g}	144.35^{k}	149.80^{e}	198.91^{f}	283.93^{e}	210.88^{i}	154.77^{g}	205.64^{g}	363.47^{d}	241.29^{f}
11	Skimmed milk		-	146.80^{c,d}	153.59^{f}	159.37^{g}	153.25^{i,j}	141.58^{e}	147.83^{f}	154.18^{f}	147.87^{k}	147.31^{g}	152.83^{g}	155.67^{e}	151.94^{g}
12	Skimmed milk		6	145.83^{c,d}	152.52^{f}	154.58^{g}	150.98^{j}	139.09^{e}	145.00^{f}	152.89^{f}	145.66^{k}	142.17^{g}	146.71^{g}	152.23^{e}	147.04^{g}
13	0.738	3.012	-	151.98^{c,d}	155.27^{f}	161.64^{g}	156.29^{i}	154.53^{e}	162.84^{f}	168.88^{f}	162.08^{j}	158.47^{g}	163.62^{g}	167.75^{e}	163.28^{g}
Mean				149.92	397.34	432.08		328.40	369.11	449.52		342.37	390.09	430.17	

Means in the same column with a common letter are not significantly different ($p \leq 0.05$)

Source	Df	MSS		
		63°C	73°C	83°C
A(Time)	2	923265.40*	148154.10*	75349.46*
B (Samples)	12	397856.90*	466670.90*	490714.10*
AXB	24	65302.960*	12599.540*	7006.292*
ERROR	78	8.769	19.865	1003.519

*significant at $p \leq 0.05$

Table 2: HMF concentration at temperatures above 100°C

Sl. No.	Treatments (%) Whey Protein	Casein	Sugar	110°C 30 min	40 min	50 min	Mean	116°C 15 sec	30 sec	45 sec	Mean	121°C 15 sec	20 sec	25 sec	Mean
1	0	0	0	0.01^{f}	0.01^{f}	0.01^{h}	0.01^{k}	0.01^{i}	0.01^{h}	0.01^{g}	0.01^{m}	0.01^{g}	0.01^{h}	0.01^{g}	0.01^{m}
2	0	0.5	6	377.31^{e}	599.39^{e}	871.33^{e}	616.22^{h}	766.64^{e,f}	882.85^{d}	1061.70^{d}	903.73^{g}	795.84^{e}	833.27^{f}	899.48^{f}	842.86^{k}
3	0	1	6	630.18^{c}	884.14^{c}	987.44^{d}	833.92^{e}	984.32^{b,c}	1046.90^{b,c}	1133.04^{d}	1054.75^{e}	902.13^{d}	1099.67^{c,d}	1198.26^{c,d}	1066.69^{f}
4	0.5	0	6	717.49^{c}	794.74^{c,d}	884.40^{d,e}	798.88^{f}	727.41^{f}	733.78^{f}	1066.52^{d}	842.57^{h}	732.62^{e}	932.48^{e,f}	1014.96^{e,f}	893.35^{j}
5	0.5	0.5	6	633.33^{c}	1246.24^{b}	1295.01^{c}	1058.20^{d}	833.12^{d,e}	916.96^{d,e}	1166.42^{c,d}	972.17^{f}	934.04^{c,d}	992.48^{d,e}	1132.40^{d,e}	1019.64^{g}
6	0.5	1	6	1166.22^{b}	1448.07^{a}	1531.80^{b}	1382.03^{c}	1017.28^{b}	1133.16^{b}	1297.84^{b}	1149.42^{b}	1108.18^{b}	1232.57^{b}	1317.02^{c}	1219.26^{d}
7	1	0	6	645.74^{c}	772.29^{d}	896.40^{d,e}	771.48^{g}	983.84^{b,c}	962.72^{c,d}	1247.36^{b,c}	1064.64^{d}	1021.21^{b,c}	1199.40^{b,c}	1533.04^{b}	1251.16^{c}
8	1	0.5	6	1334.62^{a}	1474.90^{a}	1588.90^{b}	1466.14^{b}	915.29^{c,d}	1024.43^{c}	1332.67^{a,b}	1090.80^{c}	987.15^{c,d}	1216.28^{b}	1583.03^{a,b}	1262.15^{b}
9	1	1	6	1392.06^{a}	1520.30^{a}	1747.85^{a}	1553.41^{a}	1299.08^{a}	1398.97^{a}	1432.42^{a}	1376.57^{a}	1391.57^{a}	1482.89^{a}	1698.76^{a}	1524.41^{a}
10	0.738	3.012	6	331.37^{e}	566.31^{e}	627.21^{f,g}	508.29^{j}	447.37^{h}	582.82^{d,g}	699.30^{f}	576.50^{k}	568.55^{f}	998.48^{d,e}	1123.82^{d,e}	896.95^{i}
11	Skimmed milk		-	390.72^{d,e}	585.57^{e}	588.58^{g}	521.62^{j}	530.97^{g,h}	599.36^{f}	766.12^{e,f}	632.15^{j}	698.80^{e}	1049.31^{b}	1199.87^{c,d}	982.66^{h}
12	Skimmed milk		6	473.01^{d}	617.56^{e}	735.58^{f}	608.69^{h}	598.33^{g}	616.29^{f}	835.52^{e}	683.15^{i}	765.22^{e}	1199.40^{b,c}	1315.73^{c}	1093.45^{e}
13	0.738	3.012	-	417.68^{d,e}	595.37^{e}	717.11^{f}	576.72^{i}	517.62^{g,h}	498.34^{g}	698.67^{f}	571.55^{l}	549.12^{f}	699.30^{g}	982.84^{f}	743.75^{l}
	Mean			654.60	854.22	959.35		740.10	799.74	979.82		804.19	995.03	1153.79	

Means in the same column with a common letter are not significantly different (p≤0.05)

Source	Df	MSS 110 °C	116 °C	121 °C
A(Time)	2	934521.10*	607402.40*	1194971.0*
B (Samples)	12	1751184.0*	1109816.0*	1183412.0*
AXB	24	26192.000*	12440.920*	4195732.00*
ERROR	78	76.538460	3.4871800	.28205130*

**significant at p≤0.05*

Chemical analysis

HMF estimation

HMF was estimated using the procedure of Morales *et al.* (1992), with slight modification. 20 ml samples of well-mixed milk ingredients were subjected to different heat treatments and cooled rapidly in ice bath. The samples were slowly deproteinized with 10 mL of trichloroacetic acid (TCA) solution (10%, w/v), centrifuged and filtered through Whattman No.42 filter paper. Ten ml filtrate was taken in separating funnel and extracted with 20 ml ether thrice. The extracts were combined and filtered through Whattman No.1 filter paper. Three ml filtrate was taken, added with 3 ml ethanol (99.99%) and 1% resorcinol in HCl. It was mixed well and stored in dark for 30 min at ambient temperature (25±1°C) for the development of reddish pink color. The absorbance was measured at 540 nm using Spectronic-20 spectrophotometer. The concentrations of HMF in the samples were calculated from standard curve of HMF prepared using concentrations ranging from 0 to 180 mg/ml. The amount of HMF was obtained as mg/100 g using the formula:

$$\text{HMF (mg/100g)} = \frac{\text{Conc.from standard curve} \times \text{Dilution factor}}{\text{Volume taken for colour development} \times \text{Sample wt (gm)}}$$

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%.

RESULTS AND DISCUSSION

Hydroxy methyl furfural

HMF is not present in raw milk but it is formed during the analytical procedure owing to the acidic conditions and temperature used (Morales *et al.* 1995). HMF is mainly formed by heat treatment or acid degradation of sugars where Amadori products degradation is a minor route (Berg and van Boekel 1994; Morales *et al.* 1997).

The data shows that HMF concentration increases significantly with increased time of heating at a given temperature. This is attributed to the generation of more MRPs during prolonged heating. The DMRT data shows that there is no significant difference in the amount of HMF in skimmed milk (control) and simulated milk (sample 13) at the initial stage of heat treatment. But, on progression of heating time and temperature, a significant change in HMF concentration can be noticed. This may be due to the generation of more MRPs in samples containing augmented levels of milk proteins and sucrose.

However, the DMRT data of significance for antioxidant activity and HMF at elevated heat treatments are contradictory, we may say that HMF concentration is a better index of MRPs generation in the samples. Morales and Perez (2001) found the same results while experimenting on the MRP generation with sugar-amino acid model systems. They observed that the measurement of fluorescence is the better index for antiradical activity of MRPs as the maximum fluorescence in the system is related to an acceptable free radical scavenging activity. It is a well described intermediary compound of degradation products during heat treatments and can easily be analyzed (Morales *et al.* 1992).

It has been stated by Hofmann (1998) that 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. Moreover, Morales and Perez (2001) recently attempted to correlate the biological and chemical effects of MRPs with the browning rates. Similar efforts were also made by Brands *et al.* (1995).

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 indices that are closely related to it, namely antioxidant activity and HMF concentration in terms of brown color development. Both antioxidant activity and

HMF concentration increased significantly with increase in heating time at a given temperature. However, browning is a more reliable index to estimate the amount of MRPs generated during heat processing. The simultaneous application of several heat-induced parameters 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. The results revealed that temperature and duration both have a significant effect on generation of HMF which directly correlation with generation of antioxidative maillard reaction products.

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