Advance Journal of Food Science and Technology 7(2): 81-87, 2015
DOI:10.19026/ajfst.7.1271
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: August 13, 2014 Accepted: September 14, 2014 Published: January 20, 2015

Research Article

Effects of Preheat on the Flavor and Texture Properties of Yoghurt Made of Reconstituted Milk

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Abstract: Reconstituted milk replaced fresh milk has been applied in the production of yoghurt. The flavor compounds and texture profile analysis of yoghurt produced from reconstituted milk were investigated. Using SPME-GC-MS, 24 and 22 volatiles were found in reconstituted whole milk and reconstituted skim milk, while 32 and 28 volatiles were found in their yoghurt products, respectively. Levels of aldehydes, methyl ketones, alkanes olefinic aldehydes, ketenes in liquid milk and yoghurt increased with increasing the intensity of heat treatments. Two-Pentanone, 2, 3-butanedione, 2, 3-pentanedione, 3-hydroxy-2-butanone, ethyl butyrate and 2-octanone that were metabolized by starter culture organisms showed an opposite tendency. Principle component analysis for flavor impact compounds indicated that the main difference occurred among the treatments. On the other hand, firmness, adhesiveness, chewiness and viscosity of yoghurt increased with increasing the heat intensity, while springiness, cohesiveness and resilience decreased. There was a significant difference in the textural property between the reconstituted whole milk and reconstituted skim milk ($p<0.05$).

Keywords: Preheat, reconstituted milk, texture, volatiles, yoghurt

INTRODUCTION

The typical yoghurts are the products which are made of fresh milk or reconstituted milk and fermented by lactic acid starter bacteria, *Lactobacillus delbrueckii* ssp. bulgaricus and *Streptococcus salivarius* ssp. Thermophiles (Wilkins *et al*., 1986), which is well received by customers due to its nutritional functions, healthy claims, sensory pleasures. Generally fresh milk is applied to prepare yoghurt, while the application of fresh milk is a difficult question for partial countries and regions where are short of milk supply. Gradually, researchers develop yoghurt made of reconstituted milk to meet the consumption demand.

The physical, chemical and microbiological properties have changed during the processing of milk powder, such as microbiological inactivation (Lund *et al*., 2002), interaction of whey proteins with κ-casein (Wang *et al*., 2012), oxidation of milk fat (Li *et al*., 2013) and so on. In the production of yoghurt, preheat treatment also is indispensable processing step.

Limited studies are available on the flavor of yoghurt as affected by the heat treatment. Labropoulos *et al*. (1982) evaluated the sensory and instrumental flavor of yoghurt made of fresh milk and treated by ultra-high temperature (149°C for 3.3-12.0 sec) and vat process systems (82°C for 10 and 30 min) and found no significant difference. Soukoulis *et al*. (2007) reported heat treatment of milk is considered to be a critical factor for texture formation. However, flavor and textural properties of yoghurt are highly associated with the consumer acceptance although these attributes nowadays are accompanied with certain health benefits (Drake, 2007). Based on this, the aim of the study was to determine the differences of flavor and texture properties in yoghurt, which resulted from heat treatments for reconstituted milk.

MATERIALS AND METHODS

Heat treatments: Medium-heat whole milk powder and skim milk powder were obtained from a local dairy plant. The reconstituted milk (12%, w/v) were pasteurized as described by Alloggio *et al*. (2000) with some modification. One hundred and fifty milliliter of each milk sample was added to sterile flask, hermetically sealed. The flask was immersed in a boiling water bath until the desired temperature was reached and then transferred rapidly to a stirred water bath at 80°C for 15 and 30 min. Finally, milk was cooled to incubation temperature by immersing the test flasks in a stirred water bath at 0-4°C.

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Preparation of yoghurt: The heated milk was inoculated with the starter of *Lactobacillus delbrueckii* ssp. bulgaricus and *Streptococcus salivarius* ssp. thermophilus (Danisco YO-MIX, Germany) at the concentration of 0.2 g/L (w/v). The samples were incubated at 43°C for 4 h. After termination of fermentation the yoghurt was cooled to 4°C and stored at this temperature prior to analysis.

Extraction of flavor compounds: The volatiles in the headspace of liquid milk and yoghurt were obtained using Solid Phase Microextraction (SPME) with a DVB/CAR/PDMS fiber (2 cm length) (Supelco Inc., Bellefonte, PA, USA). In details, 150 mL liquid milk or 150 mL mixture of yoghurt and water (2:1) was weighed into a 250 mL vial with adding 0.5 mL aqueous solution of 4-methyl-2-pentanone (10 mg/L) as internal standard. A stirring bar was placed into the vial. The sample was allowed to equilibrate and extract at 55°C for 45 min under mild stirring with a magnetic stirrer (HJ-3, Guohua Inc., Jiangshu, China). The SPME fiber was done at 250°C for the first 2 min in the GC injector before analysis.

Determination of volatiles: The mass spectra of volatiles were obtained using the HP 6890 gas chromatograph equipped with the HP 5973 mass selective detector (Hewlett-Packard Inc., Wilmington, Delaware, USA). The interface, quadrupole and ion source temperature were kept at 250, 280 and 230°C, respectively. A DB-5 capillary column with 60 m length, 0.25 mm internal diameter, 0.25 μm phase thickness (J and W Scientific, Folsom, CA, USA) was used. The oven temperature was maintained at 40°C for 8 min, programmed to 150°C at a rate of 4°C/min, then raised at 20°C/min to 250°C and held at 250°C for 5 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min. Electron impact ionization was used at a voltage of 70 eV. The mass range was m/z 30-500.

Volatiles were identified by the combination of NIST-98 GC-MS spectrum library and the comparison of retention time under the same operating conditions. The relative levels of individual compounds were obtained using the AR value treated as the area ratio of flavor compounds and internal standard.

Texture properties of yoghurt: The Textural Profile Analysis (TPA) was based on the method described by Marshall and Rawson (1999). Each yoghurt sample was mixed until homogeneous and then analyzed on a texture analyzer (TA.XT.plus, Stable Micro Systems Ltd, Surrey, UK) with a 5 kg load cell. The measurements were conducted at 4-6°C in the compression mode using a 40-mm diameter perspex probe. The test speed was set to 1.0 mm/sec for a distance of 10.0 mm. Hardness, adhesiveness, springiness, cohesiveness, chewiness and resilience were recorded using the TPA macro in software program called Texture Expert Excede Version 1.0 (Stable Micro Systems Software). Each sample was replicated three times and reported as mean±S.D.

Viscosity of the mixed gels: The apparent viscosity was based on the method of Marshall and Rawson (1999) at the temperature of 4°C with a viscometer (LVT, Brookfield Engineering Lab. Inc., Stoughton, Mass., USA).

Statistical analysis: Analysis were duplicated for the GC-MS and replicated three times for others. One-way Analysis of Variance (ANOVA) for distinguishing the difference in textural properties of yoghurt among the treatments and Principal Component Analysis (PCA) for the difference in volatiles were carried out using PASW Statistics18.0 Software (SPSS Inc, USA).

RESULTS AND DISCUSSION

Change in volatiles of liquid reconstituted milk: Table 1 shows the volatiles of liquid milk, including the unheated and heated reconstituted whole milk (WP and HWP), unheated and heated reconstituted skim milk (SP and HSP). A total of 24 volatiles were found in WP and HWP, 22 volatiles were found in SP and HSP using SPME-GC-MS. The levels of flavor impact compounds, such as hexanal, 2-heptanone, heptanal, 2-pentyl-furan and 2-nonanone in WP and HWP were higher than SP and HSP, while nonanal showed an opposite tendency. Two, 4-Nonadienal, octanal, 4-methyl-decane, 2-octenal, 1-octanol, 3-octen-2-one, 3, 5-octadien-2-one, 2, 6-dimethyl-undecane were only found in WP samples. The flavor impact compounds of acetophenone and 2-undecanone were only found in SP samples. The levels of these volatiles in liquid milk increased with increasing the intensity of heat treatments. Pathways of methyl ketones formation were the β-oxidation of saturated fatty acids and decarboxylation of β-ketoacids, naturally present in milk (Nursten, 1997; Contarini and Povolo, 2002). Increased AR values of aldehydes and alkanes may due to the oxidation of unsaturated fatty acids at double bonds (Nursten, 1997). The higher levels of methyl ketones, aldehydes and alkanes in WP samples mainly results from the higher contents of fat which could take part in the oxidation reactions during the treatments of preheat, condensation and drying. When WP or SP was heated the oxidation reaction of fat could further react.

Therefore, the AR results of volatiles related with lipid oxidation increased with increasing the intensity of heat treatments.

The increase of 2-pentyl-furan was highly associated with Strecker degradation during the heat treatments (Shiratsuchi *et al*., 1994). The formation of acetophenone in reconstituted skim milk was from phenylalanine and methylglyoxal via the Strecker degradation (Calvo and De La Hoz, 1992). Levels of

Table 1: Volatiles in liquid reconstituted milk

			AR					
				HWP			HSP	
No.	Retention time (min)	Volatiles	WP	80/15	80/30	SP	80/15	80/30
1	12.59	Toluene	1.29	1.27	1.34	1.84	1.88	1.87
2	14.33	Hexanal	26.88	33.71	36.77	3.15	4.76	6.39
3	16.83	Chlorobenzene	-	-	-	1.04	1.11	1.06
4	17.64	Ethylbenzene	0.56	0.55	0.57	0.47	0.41	0.46
5	18.07	p-xylene	2.29	2.29	2.43	1.62	1.77	1.52
6	19.14	2-heptanone	34.09	37.21	39.23	3.22	4.88	6.52
7	19.66	Heptanal	63.07	67.28	69.39	2.28	3.61	4.77
8	22.75	Acetophenone	-	-	-	0.47	1.85	3.69
9	24.02	2, 4-nonadienal	1.62	1.69	1.78	-	-	-
10	24.10	2-pentyl-furan	1.48	1.52	1.56	0.56	1.04	1.49
11	24.18	Trimethylbenzene	-	-	-	0.55	0.62	0.57
12	24.43	Decane	1.29	1.37	1.42	0.74	0.83	0.89
13	24.50	Octanal	13.05	13.83	14.87	-	-	-
14	25.45	4-methyl-decane	0.43	0.49	0.56	-	-	-
15	25.82	D-limonene	0.48	0.52	0.51	0.42	0.45	0.53
16	26.01	Indane	-	-	-	0.47	0.53	0.50
17	26.17	3-octen-2-one	1.35	1.62	1.79	-	-	-
18	27.55	2-octenal	2.86	3.34	4.12	-	-	-
19	27.90	1-octanol	0.77	0.79	0.84	-	-	-
20	28.38	2-nonanone	8.49	14.14	16.08	2.39	3.83	7.11
21	28.47	3, 5-octadien-2-one	2.54	2.58	2.79	-	-	-
22	28.72	Undecane	0.40	0.57	0.68	0.28	0.42	0.54
23	28.89	Nonanal	0.75	0.87	1.09	4.81	9.32	17.46
24	32.35	Naphthalene	-	-	-	1.17	1.22	1.12
25	32.63	Dodecane	0.49	0.64	0.69	0.84	0.95	1.07
26	33.16	2, 6-dimethyl-undecane	0.40	0.45	0.53	-	-	-
27	35.99	2-undecanone	-	-	-	0.58	0.93	1.32
28	36.17	Tridecane	0.25	0.37	0.49	0.27	0.32	0.35
29	37.71	Decanoic acid	0.47	0.42	0.35	0.42	0.35	0.21
30	38.42	Tetradecane	0.73	0.67	0.83	0.22	0.29	0.34

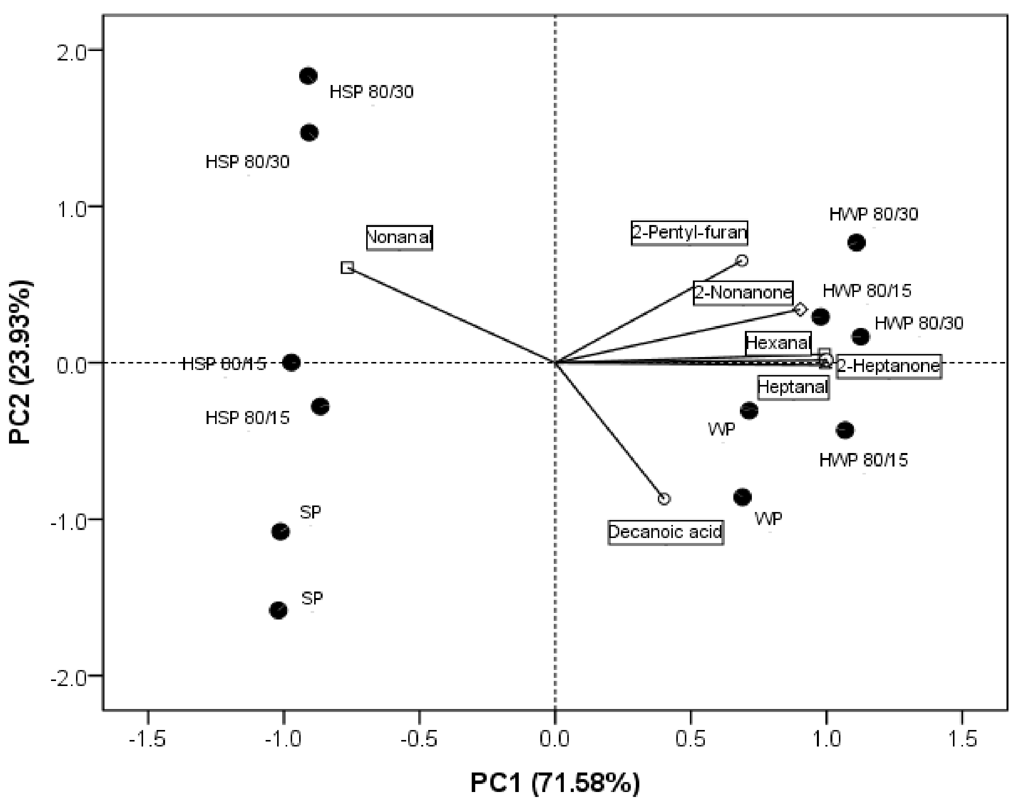

Fig. 1: Principal component biplot of volatiles in liquid milk

decanoic acid had a slight tendency to decrease with increasing the heat intensity due to the low volatility. Toluene, ethylbenzene and *p*-xylene probably came from the degradation of milk carotene. D-Limonene was probably transferred from forages (Contarini *et al*., 1997; Garde *et al*., 2005). Based on that, changes in these volatiles were not found.

PCA was performed for the selected volatiles. Figure 1 shows the principle component biplot in liquid reconstituted milk from different heat treatments. Seven volatiles, that is, hexanal, 2-heptanone, heptanal, 2-pentyl-furan, 2-nonanone, nonanal and decanoic acid were introduced as the test set. A large variation was observed in levels of volatiles between WP samples (PC 1>0) and SP samples (PC 1<0). A distinct tendency to increase except decanoic acid in PC 2 was performed for reconstituted milk with the increasing intensity of heat treatment. This diagram also shows the distinctive position of HSP because of its higher levels of nonanal, 2-pentyl-furan, 2-nonanone and lower levels of decanoic acid compared with SP.

Changes in volatiles of yoghurt: Table 2 shows the volatiles of yoghurt. Thirty two and 28 volatiles were found in yoghurt prepared from heated reconstituted whole milk (labeled as Y-HWP) and in yoghurt prepared from reconstituted skim milk (labeled as Y-HSP), respectively. The flavor impact compounds of yoghurt seemed to belong to diketones, methyl ketones, aldehydes, fatty acids, alkanes, terpenes, benzenes, olefinic aldehydes, ketenes, furan and alcohol. The number of volatiles detected in this study was less than previous studies due to different flavor separation techniques (Hettinga *et al*., 2008; Ott *et al*., 1997).

Two, 3-Butanedione and 2, 3-pentanedione are known to be produced by chemical decarboxylation of

Table 2: Volatiles in yoghurt prepared from reconstituted milk

			AR			
			YHWP		YHSP	
No.	Retention time (min)	Volatiles	80/15	80/30	80/15	80/30
1	5.84	2-pentanone	1.14	0.41	1.51	0.78
2	5.96	2, 3-butanedione	2.36	0.78	1.20	0.75
3	9.11	2, 3-pentanedione	9.74	3.94	5.33	2.85
4	9.93	3-hydroxy-2-butanone	2.24	1.68	4.47	3.34
5	12.59	Toluene	1.38	1.41	2.11	2.07
6	14.33	Hexanal	0.79	0.98	0.68	0.42
7	14.49	Ethyl butyrate	1.95	0.87	2.06	0.75
8	16.83	Chlorobenzene	-	-	1.33	1.28
9	17.64	Ethylbenzene	0.57	0.62	0.48	0.50
10	18.07	p-xylene	2.53	2.58	1.59	1.49
11	19.14	2-heptanone	44.83	45.73	8.66	11.62
12	19.66	Heptanal	2.57	5.93	-	-
13	22.75	Acetophenone	-	-	1.84	4.61
14	23.65	Hexanoic acid	14.51	16.06	6.57	16.11
15	23.91	2-octanone	2.33	1.97	-	-
16	24.02	2, 4-nonadienal	1.73	1.75	-	-
17	24.10	2-pentyl-furan	1.53	1.56	0.89	1.87
18	24.18	Trimethylbenzene	-	-	0.59	0.61
19	24.43	Decane	1.85	2.03	0.91	1.23
20	24.50	Octanal	0.38	0.55	-	-
21	25.45	4-methyl-decane	0.71	0.87	-	-
22	25.82	D-limonene	0.60	0.52	0.63	0.55
23	26.01	Indane	-	-	0.47	0.53
24	26.17	3-octen-2-one	1.58	1.72	-	-
25	27.55	2-octenal	3.49	3.64	-	-
26	27.90	1-octanol	0.89	0.84	-	-
27	28.38	2-nonanone	15.59	17.22	6.06	8.62
28	28.47	3, 5-octadien-2-one	2.42	2.66	-	-
29	28.72	Undecane	0.79	0.89	0.47	0.68
30	28.89	Nonanal	0.57	0.87	8.22	11.01
31	31.62	Octanoic acid	10.92	14.34	11.05	14.71
32	32.35	Naphthalene	-	-	1.18	1.29
33	32.63	Dodecane	1.19	1.35	1.01	1.13
34	35.99	2-undecanone	-	-	0.93	1.39
35	33.16	2, 6-dimethyl-undecane	0.94	0.88	-	-
36	36.17	Tridecane	0.76	0.77	0.32	0.41
37	37.71	Decanoic acid	1.35	1.54	3.58	6.83
38	38.42	Tetradecane	1.17	1.34	0.37	0.42

their precursors, that is, α-acetolactate and α-aceto-α-hydroxybutyrate (Ott *et al*., 1999). Two-Pentanone, 3-hydroxy-2-butanone, ethyl butyrate, hexanoic acid, 2-octanone and octanoic acid are also common metabolic products of cultures in yoghurt (Ott *et al*., 1997, 1999). The AR values of these volatiles decreased with the increasing intensity of heat treatment except volatiles of fat acids (Table 2). The results indicated the preheat treatments influenced metabolism of starter cultures.

Besides the oxidation and decarboxylation, the metabolism of starter cultures was another reason for the increasing of 2-heptanone and 2-nonanone. With the increasing intensity of heat treatment, volatiles of other aldehydes and alkanes slightly increased due to the oxidation and Strecker degradation. However, levels of aldehydes in yoghurt were lower than them in liquid milk. Especially heptanal and octanal were only detected in Y-HWP.

Figure 2 shows the principle component biplot in yoghurt from different heat treatments. Eleven flavor impact compounds were selected as the test set. Y-HWP and Y-HSP could be distinguished by PC 1. Combination of PC 1 and PC 2 allowed the inter-group of Y-HWP and Y-HSP to be distinguished.

Changes in the TPA analysis: Table 3 shows the typical TPA parameters of yoghurt produced from Y-HWP and Y-HSP. Clear differences in the textural profiles were found among different heat treatments. Either in Y-HWP and Y-HSP, hardness, adhesiveness and chewiness increased significantly with increasing the heat intensity ($p<0.05$), while springiness, cohesiveness and resilience decreased significantly ($p<0.05$). The denaturation of whey proteins could result in the formation of whey protein-coated casein micelles when milk was heated (Mottar *et al*., 1989). Vasbinder *et al*. (2003) reported the hardness of acid-induced gels depended on the amount of reactive thiol groups present after heating the milk. Therefore the denaturation and aggregation of whey protein seemed to be an important reason for the difference in textural properties of yoghurt.

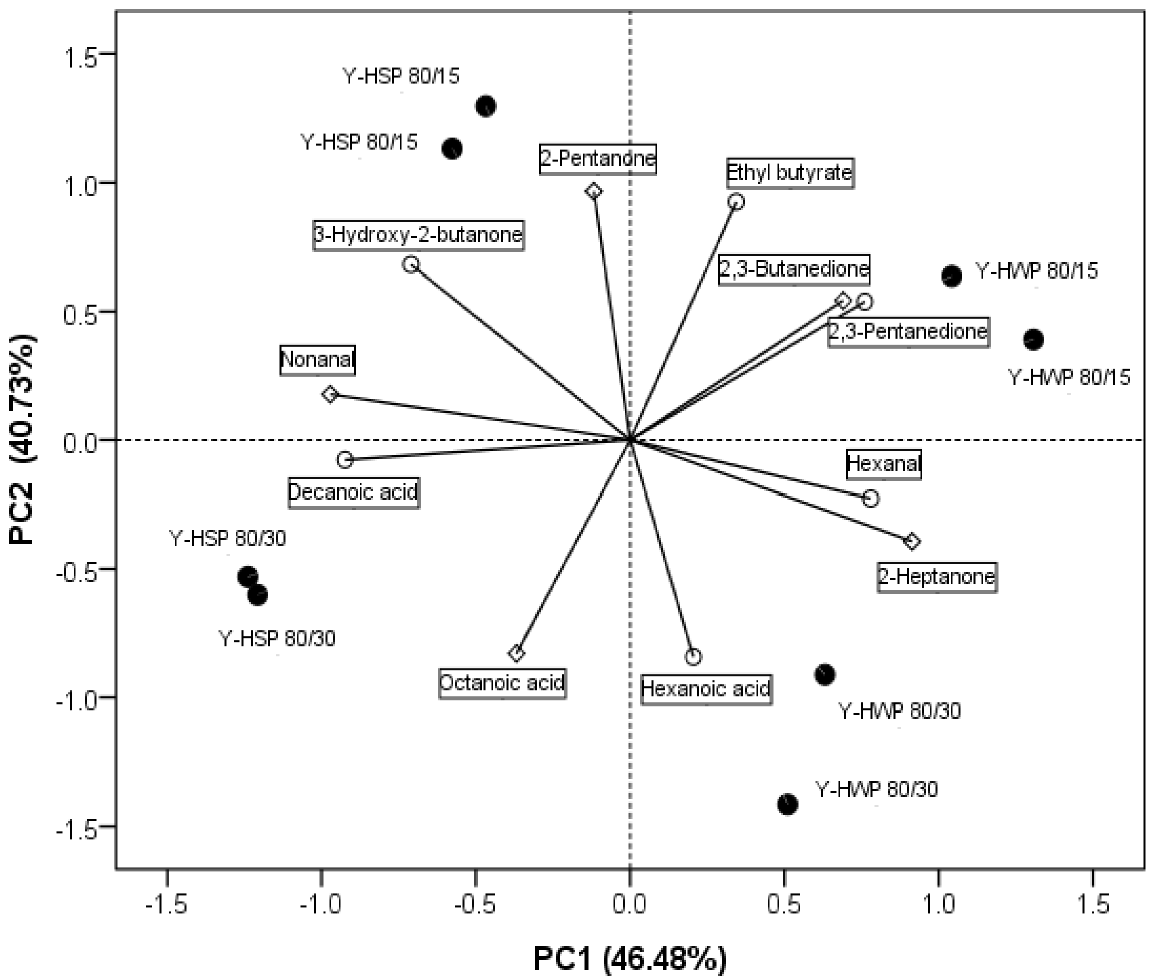

Fig. 2: Principal component biplot of volatiles in yoghurt

Table 3: Changes in typical textural parameters of yoghurt prepared from reconstituted milk

	Y-HWP		Y-HSP	
Parameters	80/15	80/30	80/15	80/30
Hardness (g)	14.148±0.501	17.745±0.286	16.629±0.714	21.556±1.445
Adhesiveness (g.s)	43.979±6.780	81.596±7.351	67.789±3.083	116.719±7.304
Springiness	0.885±0.032	0.832±0.013	0.852±0.024	0.804±0.013
Cohesiveness	0.800±0.035	0.718±0.029	0.776±0.063	0.682±0.014
Chewiness (g)	10.232±0.113	10.661±0.332	11.177±0.330	11.797±0.419
Resilience	0.311±0.019	0.072±0.008	0.087±0.004	0.031±0.005

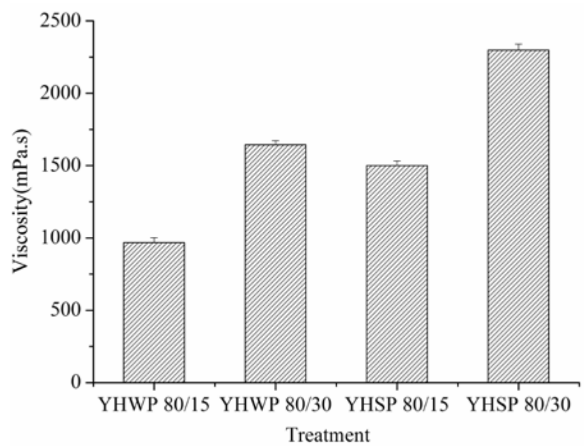

Fig. 3: Viscosity of yoghurt prepared from reconstituted milk

Change in viscosity of yoghurt: Yoghurt curd had high level of viscosity due to the fermentation of lactic acid bacteria. Figure 3 shows the changes in viscosity of yoghurt prepared from HWP and HSP. With increasing the heat intensity, viscosity of Y-HWP and Y-HSP increased significantly ($p<0.05$). Levels of viscosity in Y-HSP were higher than Y-HWP. Lucey and Singh (1997) reported heat treatment at 78°C for at least 15 min (depends on heating conditions and milk pH) results in sufficient denaturation to cause a major change in the gelation properties. High milk heat treatment leads to firmer and more viscous gels (Lucey and Singh, 1997). That was in agreement with the increasing viscosity of yoghurt in this study.

CONCLUSION

In this study, remarkable differences in flavor impact compounds existed among the reconstituted whole and skim milk, liquid milk and yoghurt. Level of volatiles generated from oxidation, decarboxylation and Strecker degradation increased with increasing the heat intensity. Common metabolic volatiles of cultures in yoghurt decreased with increasing the intensity of heat treatments. In addition, the textural properties of yoghurt between reconstituted whole and skim milk were different. Values of firmness, adhesiveness, chewiness and viscosity increased with increasing the heat intensity, while springiness, cohesiveness and resilience showed opposite tendency.

ACKNOWLEDGMENT

The authors acknowledge financial support from the National Natural Science Foundation of the People's Republic of China (project-no: 31401557) and the Introduction Talent Fund of Zhe Jiang Gong Shang University (project-no: 1110XJ2313043).

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