Advance Journal of Food Science and Technology 3(1): 16-22, 2011
ISSN: 2042-4876

Received: November 13, 2010 Accepted: December 13, 2010 Published: February, 15, 2011

Effect of Chemical Composition of Honey on Cream Formation in Honey Lemon Tea

J. Hategekimana, J.G. Ma, Y. Li, E. Karangwa, W. Tang and F. Zhong
State Key Laboratory of Food Science and Technology, School of Food Science and Technology,
Jiangnan University, Wuxi, 214122, Jiangsu, P.R. China

Abstract: The aim of this study was to assess the effect of chemical composition of honey and environmental factors such as pH, storage temperature and ionic strength on cream formation in honey lemon tea drink. The results showed that minerals, Ca^{2+} and Mg^{2+} were involved in cream formation while Fe^{2+} participated in changing color to tar black at pH 4.12 and above. Honey rich in polyphenols and proteins increased the cream to 2.51%. High proline content (0.506 mg/g) induced creaming in GH (Germany Honey) infusion while Ca^{2+} and Mg^{2+} were responsible for creaming in CH (Chinese Honey) infusion. The higher the amount of the total solids in honey, the higher the amount of cream formed. Honey lemon tea creaming can be reduced by controlling interaction between proteins and polyphenols, by pre-treatment of honey (i.e., to remove colloidal matters) or by removing Ca^{2+} or Mg^{2+}.

Key words: Cream, honey, infusion, lemon, precipitation, tea

INTRODUCTION

Tea is one of the most popular non-alcoholic beverages in the world. It has specific characteristics, such as taste, aroma, and is reported to have health effects (Rao *et al*., 2011). To consume tea drink, people like to add lemon and honey in it, which are believed to increase not only its palatability; taste and aroma, but also to enhance its health effects. The blended tea, lemon and honey commonly called "Honey Lemon Tea" may be consumed freshly hot, or consumed later after being kept at cool temperatures in refrigerator or by addition of pieces of ice.

However, like many other tea based drinks, Honey lemon tea was observed to form cream while it cools down. It is reported that creaming has not only an unattractive appearance but also it damages both its taste and color (Yin *et al*., 2009) with the loss of certain physical attributes or biological activities due to interactions with compounds responsible for sensory attributes (i.e., astringency, aroma, color, and taste) as health-promoting characteristics.

Numerous studies have reported the instances of tea cream formation in black tea, green tea or oolong tea (Liang and Xu, 2001; Xi *et al*., 2009; Yin *et al*., 2009). The mechanism of creaming in tea infusions with its characteristic polyphenolic compounds, thearubigin and theaflavin, was early identified (Jobsrtl *et al*., 2005) and this mechanism varies depending strongly on chemical composition of tea itself, production environment and storage conditions. Polyphenols (P), which have low solubility, have for a long time been held to lead creaming by coming out of solution on cooling, in complex with Teaflavins (TF) and other tea constituents, accomplishing phase separation into two immiscible phases. It has been also reported that creaming in tea is a result of various molecular types of interactions including polyphenol-caffeine complexation and polyphenol olyphenol interactions. Polyphenol-caffeine complexation is influenced by a number of gallates and hydroxyl groups of the polyphenols (Sekiya *et al.*, 1984; Chao and Chiang, 1999; Couzinet-Mossion *et al.*, 2010). Jobsrtl *et al*. (2005) and Kim (2008), their reports confirmed that Caffeine and Calcium were involved in tea creaming.

Honey and lemon have different chemical compositions that may promote creaming when mixed with tea. Having a number of flavanols, honey may contribute in creaming by supplying its flavanoids (i.e., kaempferol, 8-methoxykaempferol, quercetin 3-methyl ether, isorhamnetin, kaempferol 3-methyl ether, quercetin 3,3-dimethyl ether, quercetin 3,7-dimethyl ether, etc.) (Powell *et al.*, 1993), along with proteins, metal ions (Calcium, Iron, Magnesium etc.), minor acids and caffeine (Vandercook and Stephenson, 1966; Ferreres *et al.*, 1991; Herken *et al.*, 2009; Truchado *et al.*, 2009) while lemon juice may play a big role on the acidity (pH) of the tea infusion.

Even though honey lemon tea is believed to have health promoting characteristics and many people consume it at a regular basis, today researches focus only on creaming and identification of chemical compounds of

Corresponding Author: F. Zhong, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, 214122, Jiangsu, P.R. China. Tel: +86-13812536912; Fax: +86-510-85329081

purely green tea, oolong tea and black tea. There is no detailed report related to tea mixed with honey and lemon, neither on its creaming properties nor to other aspects. Therefore, the aim of this study was (1) to assess the effect of chemical compositions of honey on creaming of honey lemon tea, (2) influence of some processing parameters such as pH, temperature, ionic strength and (3) to identify the cause of color change in honey lemon tea infusions.

MATERIALS AND METHODS

This work was conducted from mid August end October 2010, in Jiangnan University, State Key laboratory of Food Science and Technology, Wuxi, Jiangsu Province, P.R China

Materials: Three types of honey from three different countries were used in this study. Honey samples were labeled according to their countries of origin namely CH, NH and GH to represent Chinese honey (Guan Sheng Yuan honey, Shanghai, China), New Zealand honey (Squeeze Me honey, Arataki, New Zealand) and Germany honey (Natural Bee Honey, Langnese, Germany) respectively. Lemon fruits (*Citrus limonum*) and commercially refined Lipton Black Tea bags (Lipton Yellow Label, Unilever (China) Company Ltd.) were purchased from local supermarket (Auchan, Wuxi, China). All chemicals used in this study were of analytical grade.

Methods:

Preparation of lemon juice: Washed Lemon fruits were cut in half and squeezed with citrus juicer (Tribest Citristar Citrus Juicer, model: CS-1000) into a pitcher until juice was gone. The obtained fresh juice was filtered through sieve lined with 3 layers of cheese cloth and let the juice drip through. Fresh clear lemon juice was stored in refrigerator at 4ºC before use.

Preparation of honey lemon tea infusion: Fifteen grams of tea were extracted with de-ionized water (1200 mL) at boiling temperature for 10 min with moderate agitation. The extract was filtered through a mesh sieve of 100 μm pore size and quickly cooled down to 25ºC in ice bath. The cooled extract was then divided into 4 parts containing 300 mL each. The honey was added to each part as follow: to part one was added 40.0 g of CH, to part two was added 40.0 g of NH, to part three was added 40.0 g of GH, then part four was kept as control. 14.0 mL of fresh squeezed lemon juice was added to each of the four parts. The mixtures were stirred with magnetic stirrer for 30 min then centrifuged with super speed refrigerated centrifuge (GL-20.B; Shanghai Anke Co., Ltd., Shanghai, China) (7,000 rpm, 5ºC, 15 min). For each sample, 230 mL were carefully separated from sediments and divided into 9 parts then filled into 9 appropriate pre-sterilized bottles containing 25 mL each. pH was adjusted with Sodium hydroxide and Citric acid to 2.9, 4.18 and 6.30 in triplicate. All bottles were heated at 121ºC for 10min in autoclave (Pressure steam sterilizer Model YX-260). Bottles with different pH were stored at 4ºC, 20ºC and 35ºC. Other portions of supernatants were kept for further analysis.

Total solids determination: The solids concentrations of honey, whole honey lemon tea liquor, supernatant and cream solutions were determined in triplicates by vacuum drying methods as described by Ahmed (2007). Mean values of the data obtained were used as mg/g solids concentrations.

Clarification and removal of colloidal matters from honey samples: Honey samples were clarified according to the method of Hall *et al*. (1934) with slight modification. Colloids were removed from honey by treatment with a suspension of colloidal clay, bentonite. 375 g of honey were diluted with about 300 mL of distilled water, and warmed in a water bath to 50ºC, and a 5% (v/v) suspension of bentonite was added in the proportion of 1/15 (v/v) bentonite/honey. The whole was thoroughly mixed, and kept at 30 for 75 min, after which it is filtered through a large fluted filter. The resulting clear solution was then concentrated to original honey density (79 to 86% solids by refractometer) under vacuum at a low temperature (0-2ºC).

Protein determination: Protein analysis in honey samples, cream and whole honey lemon tea was performed by Bradford Protein essay (Azeredo, 2003). The calibration equation for proteins was:
$P = 0.004xA + 0.027$ ($R = 0.987$) with P: proteins and A: absorbance.

Total sugars content: Total sugars content in honey samples were determined with Phenol Sulfuric acid method as modified by Taylor (1995). The standard curve equation was:

$$TS = 0.027x\ A - 0.03 \quad (R = 0.999)$$

where, TS: total sugars and A: absorbance.

Total and free amino acids determination: Amino acids were determined according to the method reported by Lan *et al.* (2010), using an Agilent liquid chromatograph 1100 with a UV detector operated at 338 nm. The column was ODS Hypersil (250 mm, 4.6 mm), whilst the mobile phase, consisting of 20 mM sodium acetate and 1:2 (v/v) methanol-acetonitrile, was delivered at a flow rate of 1 mL/min. The column temperature was 40ºC.

Table 1. Mineral content in mg/kg of three honey samples

Samples	N	Statistics	Fe	Cu	Mg	Ca
NH	3	Mean	3.41	2.38	10.77	201.67
		Stand dev	0.18	0.62	1.16	1.53
		Min	3.23	2.00	10.00	200.00
		Max	3.59	3.10	12.10	203.00
		Median	3.40	2.05	10.20	202.00
GH	3	Mean	4.79	3.95	24.53	258.40
		Stand dev	0.28	0.95	0.47	2.12
		Min	4.53	3.00	24.00	256.00
		Max	5.10	4.90	24.89	260.00
		Median	4.75	3.94	24.71	259.20
CH	3	Mean	34.58	2.17	16.13	258.93
		Stand dev	1.34	0.77	2.55	3.86
		Min	33.23	1.50	13.50	254.90
		Max	35.90	3.01	18.60	262.60
		Median	34.60	2.00	16.30	259.30

NH: New Zealand Honey; GH: Germany Honey; CH: Chinese Honey; N: number of trials. Fe: Iron; Mg: magnesium; Cu: copper; Ca: Calcium

Table 2. Color measurement (L*, a* and b* values)[a] of honey lemon tea kept at 20ºC for 14 days

		Day 1			Day 14			[c]Comparison			
[b]Infusions	pH	L*	a*	b*	L*	a*	b*	L*	a*	b*	E*
CH	2.90	46.61 (0.05)	12.19 (0.03)	52.03 (0.20)	54.12 (0.02)	10.32 (0.02)	55.41 (0.03)	8.81	- 1.87	3.39	9.62
	4.18	46.82 (0.24)	12.66 (0.20)	52.65 (0.36)	44.26 (0.03)	11.35 (0.03)	53.21 (0.06)	6.39	- 1.32	0.56	6.55
	6.30	29.42 (0.31)	16.55 (0.15)	45.47 (0.40)	32.41 (0.10)	23.42 (0.12)	32.41 (0.10)	2.99	6.87	- 13.06	15.05
GH	2.90	47.30 (0.22)	12.53 (0.07)	52.01 (0.12)	55.41 (0.03)	10.19 (0.04)	55.17 (0.03)	7.87	- 2.34	3.16	8.80
	4.18	45.06 (0.04)	13.50 (0.30)	51.35 (0.21)	51.35 (0.06)	19.41 (0.07)	44.83 (0.06)	- 0.23	5.92	- 6.53	8.81
	6.30	50.28 (0.07)	10.04 (0.07)	51.40 (0.36)	43.89 (0.08)	15.65 (0.04)	49.65 (0.02)	- 0.64	5.62	- 1.76	5.92
NH	2.90	39.22 (0.12)	10.59 (0.13)	38.51 (0.43)	55.17 (0.03)	13.83 (0.04)	44.26 (0.03)	5.04	3.24	5.75	8.31
	4.18	45.48 (0.56)	11.27 (0.04)	43.89 (0.36)	53.25 (0.06)	9.36 (0.09)	54.12 (0.02)	8.64	- 1.91	10.23	13.52
	6.30	44.11 (0.10)	14.97 (0.10)	45.88 (0.20)	44.86 (0.06)	19.28 (0.09)	43.89 (0.09)	- 0.22	4.32	- 2.00	4.76
Tea	2.90	46.02 (0.23)	12.27 (0.20)	52.06 (0.92)	54.35 (0.05)	12.49 (0.05)	51.35 (0.06)	5.33	0.21	- 0.72	5.38
	4.18	45.82 (0.17)	13.23 (0.26)	48.65 (0.29)	49.65 (0.02)	20.08 (0.02)	44.60 (0.10)	- 1.22	6.85	- 4.05	8.05
	6.30	51.29 (0.06)	9.96 (0.03)	49.33 (0.22)	44.60 (0.10)	11.40 (0.04)	54.35 (0.05)	3.07	1.44	5.02	6.06

Standard deviations are given in parentheses; [a]: L*, lightness (0 = black, 100 = white); +a*: red; −a*: green; +b*: yellow; −b*: blue; [b]: CH, infusion containing Chinese Honey; GH, infusion containing Germany Honey; NH, infusion containing NewZealand Honey; Tea, tea without honey; [c]: $L^* = L^*_{day14} - L^*_{day1}$ (+ L* means 14days infusion is lighter than 1day infusion, - L*means 14days infusion is darker than 1day infusion), $a^* = a^*_{day14} - a^*_{day1}$ (+ a* redder, - a* greener), $b^* = b^*_{day14} - b^*_{day1}$ (+ b* yellower, - b* bluer). E*, total color difference

Total polyphenols determination: The amount of polyphenol was measured by a photometric Folin-Ciocalteu assay as described by Xi *et al*. (2009). Standard curve was:

$$P = 0.662A + 0.068\ (R = 0.994)$$

where, P = Polyphenols and A= Absorbance.

Mineral determination: Mineral content (Magnesium, Iron and Calcium) of honey samples and cream were determined by Atomic Absorption Spectroscopy method described by Ymaz and Yavuz (1999).

Quantification of cream formed: After a storage time of 14 days, honey lemon tea infusions were centrifuged (7000 x g, 20 min at 7 ,) (eppendorf centrifuge 5810R, Westbury, NY) to allow easy removal of cream. The supernatant was kept for further chemical analysis whereas the cream sediments were carefully moved to a pre-weighed aluminum dish by washing with two 5 mL aliquots of distilled water and dried for 18 h at 90 . The dried tea cream in the aluminum weighing dishes from the oven was carefully weighed and the amount of tea cream formed was determined by calculating the difference between total solids in the initial infusion and the dried honey lemon tea cream.

Color measurement of honey lemon tea: The color of Honey lemon tea was measured at the first and 14th days of storage using a color difference meter (model: WSC-S, Hangzhou Chincan Co., Ltd., China). The color functions were calculated for 2º angle observer, through the tristimulus values X, Y, Z, taking as standard values, those of the white background (X = 75.11; Y = 79.19; Z = 85.02) and then expressed in terms of lightness (L*), red/green characteristics (a*) and blue/yellow characteristics (b*) (Kayitesi *et al.*, 2010).

Ionic strength modification: Ionic strength was modified by addition of 0.1 mL of $FeCl_2$ solution into Honey lemon tea infusion as source of Fe^{2+}. $FeCl_2$ solution was prepared by dissolving 5 mg $FeCl_2$ into 1 mL distilled water followed by filtration through Whatman No. 541 filter paper.

Statistical analysis: Analysis of variance, significant differences among means, and correlation analysis were done by use of SPSS (Version 11.5, SPSS Inc., Chicago, USA). The values were considered to be significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Influence of mineral to the cream formation: Mineral contents of the three honey samples (GH, CH and NH) are shown in Table 1, while mineral contents of cream are shown in Table 3. Analysis of mineral contents of cream

Table 3: Proteins, polyphenols and minerals contents of cream from clarified and un-clarified honey at different pH (2.90, 4.12, 4.18, 6.30) and temperatures (4, 20, 35ºC)

		Un-clear honey[1]			Clear honey[1]					Minerals[3]			
Infusion	pH	4ºC	20ºC	35ºC	4ºC	20ºC	35ºC	Proteins[1]	Polyphenols[2]	Ca	Fe	K	Mg
GH	2.90	4.03	7.02	9.92	1.10	1.70	2.31	1.29 .1	89.90 .0	549.19	85.89	–	119.73
	4.12	3.01	5.54	5.08	0.85	1.02	1.40	0.92 .3	47.30 .4	na	na		na
	4.18	3.00	5.14	4.84	0.85	1.20	1.93	0.71 .6	45.40 .5	362.31	56.01	–	94.66
	6.30	0.72	1.76	2.12	0.55	0.79	1.41	0.22 .9	34.90 .1	139.29	45.3	–	62.45
CH	2.90	5.20	8.14	10.92	1.29	2.09	3.00	1.45 .4	112.40 .9	652.92	52.32	–	118.39
	4.12	3.97	5.97	7.77	1.19	1.90	1.97	1.11 .0	77.10 .7	na	na		na
	4.18	3.89	5.61	6.00	1.09	1.89	1.93	0.81 .4	69.20 .3	437.1	42.7	–	93
	6.30	1.92	3.09	4.18	0.88	1.71	1.82	0.21 .5	52.80 .5	321.9	31.63	–	23.2
NH	2.90	3.82	7.01	8.82	1.00	2.94	3.11	0.92 .5	50.40 .3	540.21	38.45	–	105.79
	4.12	1.17	4.57	5.64	0.90	1.51	2.43	0.60 .1	45.89 .6	na	na		na
	4.18	1.20	3.49	5.56	0.90	1.39	1.99	0.53 .8	44.50 .9	240	36.3	–	90.38
	6.30	0.89	1.62	2.32	0.62	0.89	1.19	0.19 .4	40.90 .2	211.11	11.09	–	47.4

([1]): Data are expressed as mg/mL of honey lemon tea infusion; ([2]): as mg/g of honey lemon tea cream; ([3]): as mg/kg; na: not analyzed; GH: infusion containing Germany honey; CH: infusion containing Chinese Honey; NH: infusion containing New Zealand Honey

showed that among the minerals tested, Ca^{2+} and Mg^{2+} were the only minerals significantly involved in cream formation while Fe^{2+} was involved in turning infusion into dark and blackish color (Table 2). Infusions containing honey with high amount of Ca^{2+} (258.40 mg/kg in GH and 258.93 mg/kg in CH) exhibited the highest degree of creaming at different temperatures and pH (Table 3). CH and NH having difference of 57 mg/kg of Ca^{2+} content, their respective creams had a difference of 2.8 mg/kg in Ca^{2+} content which increased the total cream to 35% (1.88 mg/kg). The amount of Ca^{2+} and Mg^{2+} affected creaming differently, the higher the quantity of Ca^{2+} or Mg^{2+}, the higher the cream formed. The presence of Ca^{2+} or Mg^{2+} catalyzed natural complex reactions which exist between polyphenols and amino acids with other natural components in tea or from Honey such as caffeine, proteins and polysaccharides resulting in the formation of cream. On other hand, Ca^{2+} modifies surface charges of the particles so that it enhances cream formation (Couzinet-Mossion *et al.*, 2010). Ca^{2+} and Mg^{2+} are only minerals to affect creaming, which disagree with Kim (2008) who reported the existence of K (Potassium) as an addition to Ca^{2+} and Mg^{2+} in tea cream and both enhanced cream formation. The disagreement might be due to the difference in types of teas used. Chao *et al*. (1999) explained that Calcium ions were more easily precipitated with polyphenols than other cations in tea infusions which were the same case in this study.

Effect of Iron content on color changes: The effect of physicochemical properties of Honey and pH on color was shown on Fig. 1 as L*, a*, b* values of honey lemon tea kept at 20ºC for 14 days. In infusion containing CH, the color difference (E* = 15.05) was higher at pH 6.30 than other infusions. High Iron content in CH (34.58 mg/kg) might be the main cause of the color change of infusion to blackish at low acidic pH. This was proved when ionic strength was modified by addition of $FeCl_2$ solution as source of Fe^{2+} in honey lemon tea infusion containing GH which changed L* value from 49.31 to 25.92 at pH 6.30. This was in concordance with Merin *et al*. (1998) who reported that some samples of honey are turning the color of the tea infusion to an unappetizing tar black. Blackening effect is due to the association of Iron with polyphenolic tea compounds (Merin *et al.* 1998) by binding to polyphenols and Catechin, or epicatechin present in tea to form colored anionic complexes.

Table 4: Physicochemical contents of GH, NH and CH honey samples

	GH	NH	CH
Proteins (mg/g)	3.50	3.23	3.95
Total sugars (mg/g)	707.04	734.57	795.45
Total solids (mg/g)	3.38	3.18	5.21
Total polyphenols (mg/kg)	181.731	211.974	189.427
Total amino acids (mg/mL)	0.506	0.287	0.293

Interactions between total sugars, polyphenols, amino acids and proteins: Honey is mainly composed of carbohydrates namely glucose, fructose, sucrose, and many other minor sugars. Table 4 shows the total sugars contents of honey samples. The influence of total sugars content of honey in honey lemon tea creaming was not noticeable (data not shown).

The amount of protein and polyphenols present in honey lemon tea cream in Table 3 indicates that protein and polyphenols contributed to honey lemon tea creaming. CH had high amount of protein, 3.95 mg/g which produced 8.14 mg/g of cream at 20ºC and acidic pH. When compared to other honeys (Table 3), results showed that the difference of 0.45 mg/g of protein in honey, increased the cream by 2.51%. Precipitate formation in sample with high amount of protein can be explained by a well known interaction of hydrogen bonding between hydroxyl groups of phenolic compounds and peptide bonds of protein in forming strong insoluble polyphenol-protein complex in aqueous solution resulting in increase of haze and cloudiness (Kim, 2008). Creaming in honey lemon tea containing GH can be also associated to its high content in proline (Fig. 2 and 3). It was reported by Siebert (1999, 2006) that proteins that have high affinity for binding polyphenols are those containing

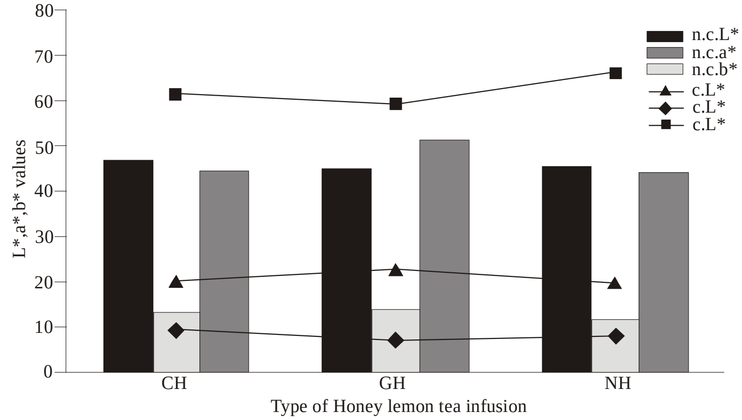

Fig. 1: Comparison between colors parameters L*, a* and b* of infusions containing un-clarified (n.c) and clarified (c) honey after storage of 14 days

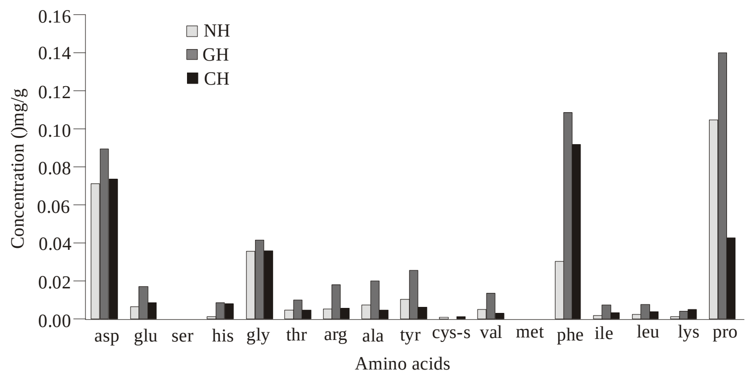

Fig. 2: Amino acids contents of GH (Germany Honey), NH (New Zealand Honey) and CH (Chinese honey)

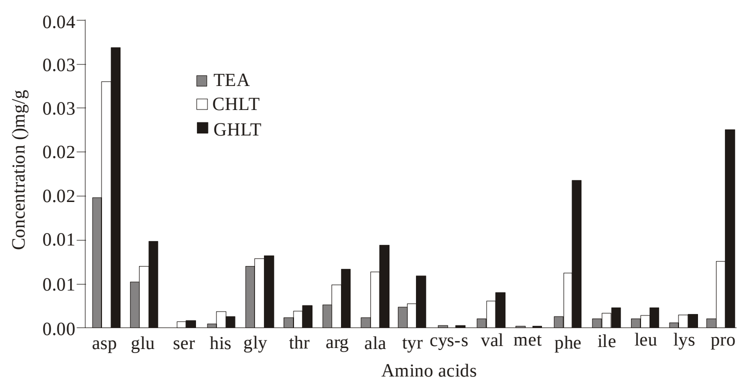

Fig. 3: Comparison of Free amino acids content in Honey lemon tea infusions containing CH (Chinese Honey) and GH (Germany Honey)

proline and the relative haze-forming activity depends on its proline content.

Effect of different pH: As pH increased from 2.90 to 6.30 there was a decrease in precipitation. At pH of 4.18 honey lemon tea was normal for few hours at room temperature then become turbid and muddy. It has been observed that at pH 6.30, honey lemon tea is blackish, and no cream formed until the 8^{th} day. The effect of pH on cream formation in honey lemon tea may be due to the degradation of some polyphenols present in tea and in honey under strong acidic conditions. Under strong acid medium, polyphenols form transient intermediate species which might be captured by polysaccharides and nucleophilic species on proteins molecules, which in turn results in cream formation (Couzinet-

Mossion *et al*., 2010). Kim (2008) reported that the precipitation at low pH was associated with hydrogen bonds as they were lowered by reduced pH and oxidation results in increased tea creaming in tea infusion.

The other driving force of tea creaming is hydrophobic interaction. When pH was elevated from 2.90 to 6.30, the hydrophobic interaction decreased resulting in precipitate formation. Another driving force of precipitation in honey lemon tea may be explained by the fact that proteins at or near their isoelectric point (pI) of pH ranging from 4 to 6 for most proteins, their negative and positive charges cancel and the net primary charge of a protein becomes zero, then repulsive electrostatic forces are reduced and the dispersive forces predominate. The dispersive forces will cause aggregation and precipitations.

Effect of total solids and colloidal matters: The effect of total solids and colloidal matters was described in Table 3 and 4. The results revealed that the quantity of total solids in original honey affected the quantity of cream formed. For example, infusion made from CH with 5.21 mg/g total solids has 5.61 mg/mL of cream at pH 4.18 and 20ºC while NH with 3.18 mg/g total solids has 3.49 mg/mL of cream at the same conditions. As long as honey lemon tea infusions stay undisturbed, solids will tend to sediment or to settle at the bottom of container due to their high molecular weight.

On their side, colloidal matters in honey are very small and are not affected by ordinary straining or filtration. In normal floral honey, they are kept in suspension by virtue of positive electric charges carried by them. This causes them to repeal each other and thus remain in suspension.

At pH below 4.18 and 20ºC, infusions containing un-clarified honey have shown high degree of creaming compared to the infusions containing cleared honeys (Table 3). The colloidal suspended particles in honey which are mainly the protein materials, enzymes (invertase, catalase and diastase), wax particles, pollen grains, silica, and other extraneous matter (Root *et al*., 2005), lost their electric charges in high acidic medium causing them to flocculate. This was in concordance with Root *et al*. (2005) who reported that the removal of electrical charges by adjusting the acidity, or by addition of correct amount of a colloidal suspension (such as bentonite) carrying opposite electrical charges, cause a tendency to flocculate and settle out.

CONCLUSION

In conclusion, this work showed that honeys from different sources have contributed on creaming in honey lemon tea depending on their main chemical components. Results affirmed that the main components of honey that influence in honey lemon tea cream formation are minerals especially Ca^{2+} and Mg^{2+}, proteins and polyphenols through the complex reactions catalyzed by metal cations. Amount of proline, total solids and colloidal matters play a great role in creaming. Honey lemon tea creaming may therefore be reduced by controlling interaction between proteins and polyphenols (i.e., increasing the solubility of the polyphenols) or by removing Ca^{2+} or Mg^{2+}. To understand the mechanism by which honey contributes to honey lemon tea creaming and driving forces of tea creaming, may help to develop a procedure to minimize creaming in honey lemon tea.

ACKNOWLEDGMENT

This work was financially supported by 111 Project-B07029.

REFERENCES

Ahmed, J., S. Prabhu, G. Raghavan and M. Ngadi, 2007. Physico-chemical, rheological, calorimetric and dielectric behavior of selected indian honey. J. Food Eng., 79(4): 1207-1213.

Azeredo, L., M.A.A. Azeredo, S.R. de Souzza and V.M.L. Dutra, 2003. Protein contents and physicochemical properties in honey samples of apis mellifera of different floral origins. Food Chem., 80(2): 249-254

Chao, Y.C. and B.H. Chiang, 1999. Cream formation in a semifermented tea. J. Sci. Food Agric., 79(12): 1767-1774.

Couzinet-Mossion, A., S. Balayssac, V. Gilard, M. Malet-Martino, M. Potin-Gautier and P. Behra, 2010. Interaction mechanisms between caffeine and polyphenols in infusions of camellia sinensis leaves. Food Chem., 119(1): 173-181.

Ferreres, F., F.A. Tom -Barber , M.I. Gil and F. Tom-Lorente, 1991. An hplc technique for flavonoid analysis in honey. J. Sci. Food. Agric., 56: 49-56.

Hall, H.H. and L.E. Lothrop, 1934. The use of clarified honey in culture media. J. Bact., 27: 349-355.

Herken, E.N., O. Erel, S. Guzel, H. Celik and S. Ibanoglu, 2009. Total antioxidant, phenolic compounds, and total oxidant status of certified and uncertified turkey's honeys. Int. J. Food Prop., 12(3): 461-468.

Jobsrtl, E., J.P.A. Fairclough, A.P. Davies and M.P. Williamson, 2005. Creaming in black tea. J. Agric. Food Chem., 53: 7997-8002.

Kayitesi, E., K.G. Duodu, A. Minnaar and H.L.D. Kock, 2010. Sensory quality of marama/sorghum composite porridges. J. Sci. Food Agric., 90: 2124-2132.

Kim, Y., 2008. Factors influencing antioxidant phytochemical stability of teas. A Dissertation of Doctor of Philosophy, Texas A&M University, Texas.

Lan, X.H., P. Liu, S.Q. Xia, C.S. Jia, D. Mukunzi, X.M. Zhang, W.S. Xia, H.X. Tian and Z.B. Xiao, 2010. Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose-soybean peptide system: Further insights into thermal degradation and cross-linking. Food Chem., 120(4): 967-972.

Liang, Y.R. and Y.R. Xu, 2001. Effect of pH on cream particle formation and solids extraction yield of black tea. Food Chem., 74: 155-160.

Merin, U., S. Bernstein and I. Rosenthal, 1998. A parameter for quality of honey. Food Chem., 63: 241-242.

Powell, C., M.N. Clifford, S.C.F. Opie, M.A. Robertson and C.L. Gibson, 1993. Tea cream formation: The contribution of black tea phenolic pigments determined by hplc. J. Sci. Food Agric., 63: 77-86.

Rao, L., K. Hayat, Y. Lv, E. Karangwa, S. Xia, C. Jia, F. Zhong and X. Zhang, 2011. Effect of ultrafiltration and fining adsorbents on the clarification of green tea. J. Food Eng., 102(4): 321-326.

Root, A.I. and E.R. Root, 2005. The abc and xyz of bee culture. Kessinger Publishing. Whitefish.

Sekiya, J., T. Kajiwara, T. Monma and A. Hatanaka, 1984. Interaction of tea catechins with proteins: Formation of protein precipitate. Agric. Biol. Chem., 48(8): 1963-1967.

Siebert, K.J., 1999. Effect of protein-polyphenol interactions on beverage haze, stabilization, and analysis. J. Agric. Food Chem., 47(2): 353-362.

Siebert, K.J., 2006. Haze formation in beverages. LWT - Food Sci. Technol., 39(9): 987-994.

Taylor, K.A.C.C., 1995. A modification of the phenol sulfuric-acid assay for total carbohydrates giving more comparable absorbances. Appl. Biochem. Biotech., 53: 207-214.

Truchado, P., F. Ferreres and F.A. Tomas-Barberan, 2009. Liquid chromatography-tandem mass spectrometry reveals the widespread occurrence of flavonoid glycosides in honey, and their potential as floral origin markers. J. Chromatogr., 1216 (43): 7241-7248.

Vandercook, C.E. and R.G. Stephenson, 1966. Lemon juice composition. Identification of the major phenolic compounds and estimation by paper chromatography. J. Agric. Food Chem., 14: 450-454.

Xi, J., D. Shen, S. Zhao, B. Lu, Y. Li and R. Zhang, 2009. Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. Int. J. Pharm., 382(1-2): 139-143.

Yin, J., Y. Xu, H. Yuan, L. Luo and X. Qian, 2009. Cream formation and main chemical components of green tea infusions processed from different parts of new shoots. Food Chem ., 114 (2): 665-670.

Ymaz, H. and O. Yavuz, 1999. Content of some trace metals in honey from south-eastern anatolia. Food Chem., 65: 475-476.