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

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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-Mossier 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...
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 Citistar 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 30ป 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 (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.
The color 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).

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).

Ionic strength modification: Ionic strength was modified by addition of 0.1 mL of FeCl2 solution into Honey lemon tea infusion as source of Fe2+. FeCl2 solution was prepared by dissolving 5 mg FeCl2 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 ≤ 0.05.
When ionic strength was modified by addition of FeCl2 infusion to blackish at low acidic pH. This was proved mg/kg might be the main cause of the color change of than other infusions. High Iron content in CH (34.58 mg/kg) might be the main cause of the color change 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 FeCl2 solution as source of Fe2+ 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 3: Provinces, 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)**

<table>
<thead>
<tr>
<th>Infusion</th>
<th>pH</th>
<th>4ºC</th>
<th>20ºC</th>
<th>35ºC</th>
<th>4ºC</th>
<th>20ºC</th>
<th>35ºC</th>
<th>Proteins¹</th>
<th>Polyphenols²</th>
<th>Ca</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>2.90</td>
<td>4.03</td>
<td>7.02</td>
<td>9.92</td>
<td>1.10</td>
<td>1.70</td>
<td>2.31</td>
<td>1.29</td>
<td>89.90</td>
<td>549.19</td>
<td>85.89</td>
<td>-</td>
<td>119.73</td>
</tr>
<tr>
<td>CH</td>
<td>4.12</td>
<td>3.01</td>
<td>5.54</td>
<td>5.08</td>
<td>0.85</td>
<td>1.02</td>
<td>1.40</td>
<td>0.92</td>
<td>47.30</td>
<td>362.31</td>
<td>56.01</td>
<td>-</td>
<td>94.66</td>
</tr>
<tr>
<td>NH</td>
<td>4.12</td>
<td>3.00</td>
<td>5.14</td>
<td>4.84</td>
<td>0.85</td>
<td>1.20</td>
<td>1.93</td>
<td>0.71</td>
<td>45.40</td>
<td>652.92</td>
<td>52.32</td>
<td>-</td>
<td>62.45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infusion</th>
<th>pH</th>
<th>4ºC</th>
<th>20ºC</th>
<th>35ºC</th>
<th>4ºC</th>
<th>20ºC</th>
<th>35ºC</th>
<th>Proteins¹</th>
<th>Polyphenols²</th>
<th>Ca</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>6.30</td>
<td>0.72</td>
<td>1.76</td>
<td>2.12</td>
<td>0.55</td>
<td>0.79</td>
<td>1.41</td>
<td>0.22</td>
<td>34.90</td>
<td>139.29</td>
<td>45.3</td>
<td>-</td>
<td>62.45</td>
</tr>
<tr>
<td>CH</td>
<td>4.12</td>
<td>3.97</td>
<td>5.97</td>
<td>7.77</td>
<td>1.19</td>
<td>1.90</td>
<td>1.97</td>
<td>1.11</td>
<td>77.10</td>
<td>477.1</td>
<td>42.7</td>
<td>-</td>
<td>62.45</td>
</tr>
<tr>
<td>NH</td>
<td>4.12</td>
<td>3.89</td>
<td>5.61</td>
<td>6.00</td>
<td>1.09</td>
<td>1.89</td>
<td>1.93</td>
<td>0.81</td>
<td>69.20</td>
<td>437.1</td>
<td>42.7</td>
<td>-</td>
<td>62.45</td>
</tr>
</tbody>
</table>

(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

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

<table>
<thead>
<tr>
<th></th>
<th>GH</th>
<th>NH</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins (mg/g)</td>
<td>3.30</td>
<td>3.23</td>
<td>3.95</td>
</tr>
<tr>
<td>Total sugars (mg/g)</td>
<td>707.04</td>
<td>734.57</td>
<td>795.45</td>
</tr>
<tr>
<td>Total solids (mg/g)</td>
<td>3.38</td>
<td>3.18</td>
<td>5.21</td>
</tr>
<tr>
<td>Total polyphenols (mg/kg)</td>
<td>181.73</td>
<td>211.97</td>
<td>189.42</td>
</tr>
<tr>
<td>Total amino acids (mg/mL)</td>
<td>0.506</td>
<td>0.287</td>
<td>0.293</td>
</tr>
</tbody>
</table>

**Effect of Iron content on color changes:** The effect of physicochemical properties of Honey and pH on color was shown in 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 FeCl2 solution as source of Fe2+ 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.

**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...
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 8th 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 (Couzinnet-
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 remain in suspension.

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 unclarified 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 Ca²⁺ and Mg²⁺, 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 Ca²⁺ or Mg²⁺. 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**

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**REFERENCES**


