# Rheological Properties and Sensory Characteristics of Green Tea Yogurt during Storage

Shabboo Amirdivani<sup>\*1,2</sup> and Ahmad Salihin Hj Baba<sup>1</sup>

<sup>1</sup>Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia <sup>2</sup> Applied Science and Technology-Alifard University, Hashtgherd, Iran \* <u>sh.mohajer1979@yahoo.com</u>

Abstract: Malaysian and Japanese green tea extract (2%) was added to a yogurt (MGTY and JGTY). Its dynamic rheological and sensory properties were evaluated and compared to those of a plain yogurt (PY) without the added green tea extract during 28 day storage at 4 °C. The plain yogurt exhibit a more compacted casein micelle aggregates network than that of green tea yogurts which was more runny and showed low viscosity attributed to the green tea extracts. The rheological analysis showed that the green tea yogurts had lower storage modulus modulus (G') and loss modulus (G") values in the linear viscoelastic region than the plain yogurt. Sensory evaluation showed that the green tea yogurts had significantly (p< 0.05) higher scored aroma and overall acceptability than plain yogurt. Incorporation of green tea extracts increased WHC in green tea yogurts contributed to lower synersi and higher total solid content than that plain yogurt during storage at 4 °C indicating both green tea yogurts may constitute a functional food with commercial application.

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### Introduction

Yogurt and related fermented dairy products have considerable economic importance worldwide owing to their high nutritional values. For instance, most dairy products usually contain substantial amount of highly bioactive compounds produced as a result of enzymes breakdown of milk proteins [1]. This strategic property can be further diversified by adding nutraceutical (nutritional and pharmaceutical) ingredients such as plant extracts rich in phytochemicals to the yogurt [2]. In the present study green tea rich in polyphenolic compounds was used to fortify vogurt, thus making herbal-vogurt a new fermented food category targeting consumers with variety health issues associated with beneficial effects of green tea consumption [3]. However, the presence of green tea affected the fermentation of milk resulting in increased acidification and proteolysis. These are expected to alter the coagulation of milk protein and subsequently the modification of textural and rheological properties of vogurt. Determining the consistency of the vogurt gel network by rheological methods is a challenge because the structure can be partly damaged in the act of sample preparation. In addition factors related to vogurt formation i.e a) the heat treatment/homogenization of the milk base (casein/whey protein ratio), b) the starter culture, and c) technological influences such as temperature, pressure, valves and the shear history are also known to be an important determinant of yogurt structure [4]. The starter culture in turn may also be influenced by the presence of green tea. The objectives of the present study were to determine the influence of green tea supplemented yogurt on its sensory characteristics and various physical and rheological properties, WHC, whey separation and total solid values during storage.

#### Materials and Methods Rheology measurements

The viscoelastic properties of the vogurt samples were determined by small amplitude oscillatory measurement (SAOM) using Bohlin CVO-R rheometer (Malvern Instrument UK). The rheometer was equipped with a temperature and moisture regulating hood and cone-plate geometry  $(20.0 \pm 0.1^{\circ}C)$ , Cone type: 40/40 mm cone Plate and a geometry gap of 0.150 mm). The temperature of the system was regulated by a viscotherm VT2 circulating bath and controlled (-40 to 180°C, Peltier Plate System, Bohlin Instrument Ltd.) acts as temperature controller. The data of the rheological measurements were analyzed with the supporting software. All the samples were gently stirred with a plastic spoon prior to loading a portion of the sample on the inset plate. The samples were subjected to a frequency sweep test using a frequency ramp from 0.001 to 10 Hz at a controlled strain mode ascertain the viscoelastic properties.

### **Oscillation measurement**

Amplitude sweep was first performed in a controlled strain mode with applied strain at a fixed unit of range 0.0005 to 0.1 at a constant frequency of 0.5Hz.

# Viscometry measurement

Viscometry measurement was performed by controlling shear rate from 0.01-80s<sup>-1</sup>. Viscosity is measured in an increasing shear rate manner. All the measurements were performed on yogurts subjected to 1, 7, 14, 21 and 28 days of refrigerated (4°C) storage.

# Syneresis

Syneresis was carried out using the siphon method as described by Amatayakul, Sherkat, & Shah [5] A cup of yogurt was weighed correct to 4 decimal places and the container was then positioned at an angle of  $45\square$  for 2 hours at  $4\square$ C. The whey accumulated was removed from the yogurt surface by using a syringe and the cups were then re-weighed. Syneresis was reported in terms of the percentage of whey lost using the following formula:

Syneresis (%) = (whey lost / sample weight) x 100%

# Water holding capacity (WHC)

Water hold capacity was performed as described by Minto et al. (2010). The milk-mixture aliquots (30ml) were placed in pre-weighed 50ml centrifuge tubes (Wtempty tubes; Oak Ridge Centrifuge Tubes). The tubes were then placed in an incubator to allow yogurt fermentation 41 C to occur until it reached pH4.5. The changes in pH were monitored using tracer container, i.e. another test tube containing milk with the same treatment conditions. The test tubes containing yogurts were weighed (Wt<sub>sample</sub>) and subsequently placed in the refrigerator  $(4\square C)$  followed by centrifugation (9500 rpm, 40 minutes,  $10\Box C$ ) the next day. Separated supernatant was discarded and tubes were reweighed (Wt<sub>nellet</sub>). The weight of the pellet was determined by weight differences. Water holding capacity was reported in terms of percentage of pellet weight by using the following formula:

WHC (%) = [(Wtpellet - Wtempty tubes)/ (Wtsample - Wtempty tubes)] x 100%

### Total solid

The total solid was determined as described by Hooi et al. (2004). Approximately 3g of yogurt samples were placed in pre-weighed aluminum pans ( $Wt_{empty pan}$ ) and these were weighed ( $Wt_{before drying}$ ) and subsequently placed into atmospheric oven at 100 $\Box$ C for 5 hours drying. The dried samples were cooled down to room temperature in a desiccator containing cobalt (II) chloride anhydrous. The aluminum pans containing dried yogurt samples were re-weighed ( $Wt_{after drying}$ ) and the total solid was reported in terms of percentage of yogurt solids using the following formula:

Total solids (%) = [ (Wtafter drying - Wtempty pan)/ (Wtbefore drying - Wtempty pan) ] x100%

# Sensory analysis

The consumer acceptability studies were carried out using organeoleptic evaluation of vogurt by a jury of 10 panelists (mean age 25 years old). Six parameters i.e. flavour, appearance, color, texture, aroma and overall appreciation were evaluated using a sensory rating scale of 1-10 (1 for extremely dislike, to 10 for extremely like). The panels recognized the yogurt only by codes. Each panel was requested to rinse their mouth by drinking mineral water after assessing each yogurt. The flavor was assessed by the estimation of acidity developed by specific lactic acid bacteria in the samples. A strict protocol was imposed to panelists to minimize variability. At each session, subjects tasted samples of chilled (4°C) yogurt (5 g). They were asked to keep the vogurt in the mouth for 12sec prior to swallowing. The subjects tasted samples of yogurt in the most natural possible way by keeping their mouth closed and swallowing the product. The yogurt samples were presented in random order. A small period of several minutes rest was required between tasting samples.

# Statistical analysis

A total of three separate experiments was carried out and assays were performed in triplicate. Data were expressed as mean  $\pm$  standard deviation and the data were analyzed using SPSS 19.0 (Chicago, IL, USA) for Windows. General Linear Model procedures and Tukey test for means comparison were used for determining significant difference at p < 0.05.

### **Results and Discussion**

# Effects of green tea on rheological characteristics of refrigerated yogurt

For rheological measurements, vogurts were stirred, as consumers usually do before consumption. prior to sampling. This is the most used method to perform viscometry or oscillation tests on vogurt probably because it is difficult to find and standardize a mechanical and reproducible method to stir yogurts without breaking substantial amount of their structure [6]. Manual slow stirring is regarded as the most effective way to preserve yogurt structure and at the same time allow preparation of yogurt sample that can be measured in a rheometer equipped with parallel plate geometry [6], suggested that when the vogurt network is very weak, sedimentation of casein aggregates occur, and that the syneresis triggered by this agglomeration leads to the formation of a depleted layer at the upper surface of the sample. When oscillatory measures are to be taken in a horizontal geometry, this layer causes slippage of the moving element, thus giving rise to an apparent reduction in modulus.

### **Apparent viscosity**

The apparent viscosity of plain and green tea yogurts is shown in Figure 1. All three yogurts showed shear thinning properties, demonstrated as decreased viscosity as the rate of shearing increased (at higher shear rate the viscosity of the yogurt decreases making it more runny). If time is considered as variable factor, the fluid may show rheopectic behavior. This is generally due to a reversible change in the structure of the material with time under shear, with a limiting viscosity ultimately being approached [7]. Green tea yogurts showed a significant decrease in viscosity with time as compared to plain yogurt at all storage periods studied. On the 1st day, the initial apparent viscosity of plain yogurt (291.3 Pas) was higher than MGT and JGT-yogurts (86.41 and 76.98 Pas; p<0.05; Figure 1). Although refrigerated storage had significant (p<0.05) effect on initial apparent viscosity of green tea-yogurts (86.41-107.8, 76.98-115.7 Pas for MGT and JGT-yogurts respectively; Figure 2, 3 and 4), but caused adverse effects on viscosity in plain yogurt as demonstrated by the reduced viscosity on day 28 (230.8 ±1. 7 Pas) compared to that in fresh yogurt (291.3 ±1. 64 Pas).



Figure 1. Viscosities of fresh plain, Malaysia green tea and Japanese green tea-yogurt



Figure 2. Viscosity for plain yogurt during storage



Figure 3. Viscosity of Malaysian green tea yogurt during storage



Figure 4. Viscosity of Japanese green tea yogurt during storage

#### Dynamic rheological measurements Frequency sweep

Yogurt is a viscoelastic material, so that its rheological behavior can be described by two parameters; namely, the storage (G'; elasticity) and the loss (G", viscosity) module [8,9]. G' is a measure of the energy stored and subsequently released per cycle of deformation per unit volume. It is the property that relates to the molecular events of an elastic nature. G" is a measure of the energy dissipated as heat per cycle of deformation per unit volume. G" is the property that relates to the molecular events of viscous nature. Another commonly used dynamic viscoelastic property, the loss tangent (which equals G"/G') denotes relative effects of viscous and elastic components in a viscoelastic behavior.

Figures 5, 6, 7 and 8 show the viscoelastic behavior of the fresh and refrigerated plain- and green tea -yogurts during storage, which occurs in practice when samples are taken out of the refrigerator for consumption and then stored again. Elastic modulus dominating viscous modulus (G'>G") showed that yogurt is having solid behavior for all of the three yogurts type. As the frequency increases the G' and G" increases gradually. These results are in accordance to Sendra et al.[10] which showed yogurt's predominantly elastic behavior (G' > G'') over the whole range of frequencies tested (0.1-10 rad/s) and which corresponds closely to that of a true gel. G' higher than G" can be due to decreased electrostatic repulsion and increased casein-casein interactions [11]. Fresh plain vogurt showed a higher range of G" and G' than the green

tea- yogurts (31.34- 72.84, 12.73- 37.26 and 11.29 - 36.23 Pa, for PY, MGTY and JGTY respectively; p<0.05; Figure 5), which is showing the improvement of viscoelasticity of the sample due to higher solid content.

There was no significant (p>0.05) difference in the Elastic modulus and viscous modulus of plain and green tea yogurts during of storage. This indicates that the no significant change in the bonding involved in the formation of gel structure by adding green tea, which may be attributed to the function of phenolic compounds in green tea that no interacted as a crosslink between protein molecules. Therefore, in green tea yogurt, the nature of bonding did not change but the extent of cross-linking increased due to the presence of dissolved phenolic compounds.

However, on  $21^{\text{st}}$  day and  $28^{\text{th}}$  day of storage, plain yogurt showed statistically different G' and G' from that on  $1^{\text{st}}$  day,  $7^{\text{th}}$  and  $14^{\text{th}}$  day; however, the difference was very small (p>0.05). This shows an increase in solid like characteristics of the samples on storage for 2 weeks which may be due to increase in the acidity of the samples during storage.



Figure 5 Frequency sweep of fresh plain yogurt, Malaysia green tea yogurt and Japanese green tea yogurt



Figure 6 Frequency sweep for Malaysia's green tea yogurt for 1<sup>st</sup> day, 7<sup>th</sup> day, 14<sup>th</sup> day, 21<sup>st</sup> day and 28<sup>th</sup> day.



Figure 7 Frequency sweep for Japanese green tea yogurt during storage



Figure8 Frequency sweep for plain yoghurt for 1<sup>st</sup> day, 7<sup>th</sup> day, 14<sup>th</sup> day, 21<sup>st</sup> day and 28<sup>th</sup> day

### Amplitude sweep

Amplitude oscillatory rheology has been used to characterize the rheological properties of yogurt during the gel formation process (fermentation) without damaging the weak gel network. Small deformation is defined as a small relative deformation (strain or change in dimension) (e.g.  $\leq 1\%$ ), which when applied does not disrupt the development of the network structure, i.e., within the linear viscoelastic region. In this "linear" region, the dynamic moduli are independent of the applied stress or strain [12]. Figure 9 shows the values of amplitude sweep for fresh plain and green tea-yogurts. The critical strain of fresh plain yogurt was the highest (44.07 - 16.58 Pa) followed by JGTY and MGTY (27.9 - 6.95 and 24.24 - 7.69 Pa respectively) in the range of 0.0004 - 0.10 strain. The elevated critical strains (yield point) suggest that the plain yogurt had a higher resistance to deformation. The high elastic modulus (G') for plain yogurt indicates it has more solid like and the binding particles (in this case milk protein) is packed closer than those in green tea-yogurts.

While JGTY showed lower elastic modulus (G') with initial strain (0.0004) compared to plain yogurt suggested that it had lower stiffness and weaker than plain yogurts. It had a slightly higher critical strain than Malaysia green tea yogurt hence it is moderate in nature, followed by MGTY which had the lowest elastic modulus (G') with same strain compared with plain yogurt, suggested that it has less solid behavior (Figure 9). The differences in the storage modulus reflected the gelation characteristics within the different yogurts.

Figures 10, 11 and 12 showed amplitude sweep of samples during storage. PY showed the highest value of elastic modulus (G') in 7th day of storage (66.68- 28.73 Pa; Figure 10) with the lowest value on the 28th day of storage (38.76- 15.59 Pa).

Suggest that in 7 day yogurt was more solid like and the particles (proteins) are closely packed. Present of green tea to vogurt indicated no effect on vogurts during storage. The changes in values of elastic modulus (G') for both green tea vogurts were not significant .Except for JGTY in the 28th day of storage which showed the highest elastic modulus value (56.44- 23.56 Pa; Figure 12). The lower value observed in yogurts fortified with the green tea can be attributed to the production of exopolysaccharide of the green tea. The filaments of exopolysaccharides interfere with the case in network. It can be assumed that protein strand formation and protein-protein interaction is partly inhibited by the exopolysaccharides, thus reducing the rigidity of the resulting yogurt gel.



Figure 9 Amplitude sweep of fresh plain, Malaysia green tea and Japanese green tea- yogurts



Figure 10 Amplitude sweep for plain yogurt during storage



Figure 11 Amplitude sweep for Malaysian green tea yogurt during storage



Figure 12 Amplitude sweep for Japanese green tea yogurt during storage.

Effects of green tea on sensory evaluation of yogurt

The effects of adding green tea on sensory properties of yogurt samples are shown in Figure 13. Yogurt tasters play an important role in the quality assessment of samples. Yogurt tasters' parameters for evaluation included appearance, flavor, color, texture and aroma. The presence of green tea in yogurt increased yogurt appearance ( $7.1\pm1.5$  and  $6.6\pm1.3$  for MGT and JGT –yogurts respectively) compared to plain-yogurt ( $5.1\pm1.3$ ), indicating that both MGT and JGT yogurts were preferred by the panelists. Texture score was lower for MGT and JGT –yogurts ( $6.4\pm1.7$  and  $6.1\pm1.9$  respectively) compared to plain ( $7.4\pm1.7$ ). Plain yogurt on the other hand showed a low flavor score ( $5.9\pm1.5$ ) compared to MGT and JGT–yogurts ( $7.45\pm1.5$  and  $7.5\pm1.4$  respectively).

Colour has much impact on the acceptability of the green tea vogurt samples (8.48±1.8, 8.0±1.7 and 5.9±2.2 for MGTY, JGTY and PY respectively). Green tea also increased (p<0.05) the fresh yogurt score for aroma (8.9±1.3 and 8.3±1.5 for MGTY and JGTY respectively) and the overall score (8.9±1.8 and 8.9±0.8 for MGTY and JGTY respectively) compared to plain-yogurt (5.3±2.3 and 6.5±1.8 respectively). In recent years, per capita consumption of yogurt has increased drastically because many consumers associate yogurt with good health [13]. Yogurt is characterized as a fermented milk product with a refreshing flavor, a smooth viscous gel, and a slight sour taste [14]. These sensory properties in addition to appearance, flavor, texture, and overall quality offer quality control criteria [15] and thus

were used in the evaluation of yogurt. The mean sensory characters scores for all samples were 5.0 or higher. The hedonic score of 5 corresponds to moderate liking of the samples, and the hedonic score of 8 corresponds to very much liking of the samples. Both green tea yogurts were liked moderately by the consumers for their texture, which were less than considered for plain yogurt.

Since the flavour of yogurt is affected by the rate of acid production during the fermentation process [16], the high score of flavour in both green tea yogurts indicate that the addition of green tea contribute to more acid production from conversion of lactose to lactic acid. This finding is important because the addition of some lactic acid bacteria can affect flavour compounds. Sensory evaluation showed that the plain  $(5.3\pm2.3)$  and the green tea yogurts (8.9±1.3 and 8.3±1.5 for MGTY and JGTY respectively) scored significantly higher (P < 0.05) aroma measurement. Acetaldehyde was firstly reported by Pette & Lolkema [17] as the main aromatic compound in yogurt. During manufacture, production of this compound is only highlighted when a certain level of acidification is reached (pH 5.0). The maximum amount is obtained at pH 4.2 and stabilizes at pH 4.0. The production of acetaldehyde and other flavour compounds by S. thermophilus and Lb.bulgaricus occurs during yogurt fermentation and the final amount is dependent on specific enzymes which are able to catalyse the formation of carbon compounds from the various milk constituents. This result was consistent with changes in pH and titratable acidity values that is, yogurts with high acidity contained the highest amount of acetaldehyde. Since the presence of green tea to milk increased acidity of yogurts it was suggested that decomposition of aroma compounds occurred more in green tea yogurts than plain yogurt during fermentation.

Overall, significant difference was observed between green tea yogurts  $(8.9\pm1.8 \text{ and} 8.4\pm0.8$  for MGTY and JGTY respectively) and plain yogurt  $(6.5\pm1.8)$  in the scores of overall acceptability; even though no significant difference was observed for MGTY and the JGTY samples. This indicated that addition of green tea affected the overall acceptability of yogurt due to the higher score of colour, aroma as well as the acceptable flavour and appearance. Green tea yogurt has the potential to attract new yogurt consumers because the incorporation of green tea into yogurt can enhance the therapeutic value of yogurt. Green tea yogurt could provide novelty in the dairy foods market and help consumers obtain nutritious food with added health benefits.



Figure 13 Sensory analysis results of plain and green tea yogurts

# Effects of green tea on water holding capacity (WHC) of yogurt during storage

Water holding capacity (WHC) is related to the ability of the proteins to retain water within the yogurt structure [18]. The mobility of water molecules in yogurt, as reflected in WHC values, can affect yield, sensory evaluation, stability (in physical terms) and texture. In fact WHC is an essential quality parameter such that the viscosity can be increased, gel-structures can be created or the physical stability can be lengthened by changing the WHC [19]. Thus changes in WHC as a result of functional additive such as herb water extracts may modify the properties of yogurt in a predetermined manner.

In this study, WHC measurements showed significant differences between green tea and plain yogurt samples (Figure 14). The higher WHC was

obtained for green tea yogurt samples  $(30.2\pm1.2 \text{ and} 31.25\pm0.67 \%$  for MGTY and JGTY respectively p<0.05) than those obtained in plain yogurt (26.05±1.5 %) in first day which was 2.92 and 3.02% higher than that of plain yogurt. The WHC of plain and green tea yogurts increased during frist week (34.4±1.6, 33.8±1.1 and27.71±0.46% for MGTY, JGTY and PY respectively on 7<sup>th</sup> day of storage) and thereafter it deacreased (27.48±1.75, 27.75±2.2 and 23.98±0.96 % for MGTY, JGTY and PY respectively on 28<sup>th</sup> day of storage).Decrease in WHC of yogurt during storage was observed by Sahan, Yasar, & Hayaloglu [20]. Decrease WHC in yogurts during storage is partly due to the unstable gel network of

yogurts, in which the weak colloidal linkage of protein micelles cannot entrap water within its threedimensional network [21].However Parnell-Clunies, Kakuda, Mullen, Arnott, & DeMan [22] hypothesized that as the  $\beta$ -lactoglobulin interacted with  $\kappa$ -casein, more covalent bonds were formed and larger micelle sizes might cause steric hindrances; all resulting in lower WHC (covalent bonds decrease the number of charged groups present in the gel network) during storage. In yogurts, increased micelle size and increased whey-casein and casein-casein interactions lead to a more porous gel, which could retain more water [23,24].



Figure 14 Water holding capacity of yogurt by adding green tea during storage Values are mean±. SD. (n=3).

# Effects of green tea on changes of syneresis on yogurt during storage

Syneresis is defined as gel contraction that occurs concomitantly with liquid/whey expulsion and relates to the inability of the gel network to entrap all of the liquid phase. Most consumers consider syneresis to be a defect [25]. When the casein particles rearrange in the gel network, whey expulsion is spontaneous, as the gel shrinks without the application of some external force [26]. The syneresis (%) of all 3 types of fresh and stored yogurt is given in Figure 15. On the first day of storage the green tea yogurts showed the lower synersis  $(3.29\pm0.89 \text{ and } 3.26\pm0.97 \text{ \%})$  than those in plain yogurt ( $3.56\pm1.1$  %; p> 0.05). All yogurts showed an increase in the amount of syneresis up to 28 days of storage (4.13±0.3, 4.18±1.0 and 4.91±1.3%; for MGTY, JGTY and PY respectively; p < 0.05) at 4 °C. These findings are in agreement with those of [9, 27] who showed an increase in the extent of syneresis in yogurts with refrigerated storage. An increase in syneresis with storage time was observed in all

yogurts. Although the phenomena occurring during syneresis are not fully understood, it is agreed that increased syneresis with storage time is usually associated with severe casein network rearrangements [9, 28], that promote whey expulsion. Functionality of hydrocolloids in yogurt is demonstrated by their ability to bind water, interact with the milk constituents (mainly proteins), and stabilize the protein network, preventing free movement of water [29].

Whey separation is known to be related to instability of gel network and thus the loss in ability to entrap all the serum phase [26]. The rate of acidification is instrumental in whey separation of yogurt in which the faster the rate the poorer the network rearrangement during whey expulsion thereby resulting in lesser whey separation [30]. The shorter time for green tea yogurts than plain yogurts to reach pH4.5 may result in the reduction in whey separation in MGTY and JGTY as compared to plain-yogurt.



Figure 15 Effects of Green tea extract on the syneresis of yogurt

### Effect of green tea on total solid content in yogurt

The total solid (TS) content of yogurt samples showed in Figure16. It was observed from the results that the total solids content in the MGTY was in the range of 9-13 %, and JGTY was in the range of 8-13 % while PY was in the range of 7-13 %.

These TS values were much lower than those in commercial yogurts which have a typical range of 14-15% [29]. This is because yogurt prepared in the present studies was without the addition of stabilizers or milk powder which commonly practiced in the commercial preparation of set or stirred yogurt. The apparent decrease in total solids with storage time (8.72, 8.44 and 6.97 % for MGTY, JGTY and PY respectively) in day 28 may be explained by moisture loss which had an advantage of increased syneresis (Figure 14). The total solids content of the yogurt samples had a significant effect on the degree of syneresis. Reduction of free water and increasing the proportion of solids content, which occur during fermentation, are two main factors decreased rates of wheying off in the samples with high total solids. This is agreeing with the finding by Jaros, Partschefeld, Henle, & Rohm [31] who found that increase whey separation of yogurt occurred only when the total solids were decreased. In this regard the addition of green tea may be seen as advantageous in reducing the wheying off while yogurt is being kept refrigerated.



Figure16 Changes in total solid content of yogurts during refrigerated storage

# Conclusion

The addition of green tea (2 g), as a source of phytochemicals, to supplement yogurts appear to decrease viscosity behavior, elastic modulus and amplitude sweep than those found in plain yogurt but promoted avenue for increased sensory properties, with high consumer acceptability. Increased WHC in green tea yogurts contributed to lower synersis and higher total solid content. Both green teas itself are well known for their beneficial health effects, and together with yogurt they may constitute a functional food with commercial applications.

# **Corresponding Author:**

### Shabboo Amirdivani

Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia Email: <u>sh.mohajer1979@yahoo.com</u>

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