Technical Session 6
Chemistry of Quality Improvement and Value Addition
Chairman: Dr. Nigel Melican

Opening Remarks of Session Chairman Dr. Nigel Melican

Today's session is on price realization. Yesterday we discussed cost saving which is a panic reaction to an adverse situation. The long-term solution is to receive higher prices for your product. That is not possible as long tea is treated as a commodity. It must be accepted as a consumer product, which is designed for those markets that can support higher prices. Product quality and specific attributes must be designed as per the consumer requirement. There is no better way of doing that than understanding the chemistry of tea. It can then be manipulated to make a designer product that will fetch the price you want.

Comments by Dr. N.K. Jain

Prof. Tei Yamanishi is a very dedicated scientist and one of the tallest in the area of tea flavor chemistry. She was our guest scientist at IHBT in Himachal where she worked long hours with Ravindra Nath to set up equipment to study flavor of Himachal teas. She always likes to share her knowledge with the colleagues in India. She was the Guest of Honor at the 2nd International Conference held at Delhi in 1996. Initially I did not invite her considering her frail health and age of 86 years. One day I received a complaint via her student Luo Shao Jun of China. When I sent her an invitation and made a follow up call, she immediately gave her consent saying " Oh yes! I am coming". My friends, who want to improve the flavor of their tea, will derive great benefit by discussion with her. Thank you Tei for accepting to be with us today. With these few words on behalf of all of you, I welcome Prof. Tei Yamanishi, the ever-young senior citizen.

Chapter 25

CHEMICAL BASIS OF TEA QUALITY

Tei Yamanishi[1]

Introduction of Prof. Yamanishi by Session Chairman Mr. Melican

Dr. Yamanishi was born in 1916 in Kumamoto Prefecture. In 1958 she graduated from Hokkaido Imperial University and went to MIT. After graduation she worked in tea chemistry, became Assistant Professor, then Professor and in 1982 retired as Professor Emeritus. She has been an inspiration to tea biochemists around the world for many years. When I was working in a laboratory, I followed her work very closely. I first met her in Shizuoka at a tea conference 12 years ago. At an age when most of us will be retired, she is still enthusiastic to share her tea wisdom with us. I may say that she is a living tribute to the health benefits of green tea.

Tea is a unique beverage having a characteristic flavor that is one of the most important factors in determining the quality of tea. The term 'flavor' includes both taste and aroma. The characteristic taste of tea is made up of a balanced mixture of astringency, bitterness, Umami taste, and a hint of sweetness.

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STRUCTURE AND CHEMICAL COMPOSITION OF TEA LEAVES

The principal contributors of astringency and bitterness are catechins and caffeine. Umami taste is mainly due to amino acids. These compounds are contained in fresh tea leaves. As shown in Figure 1, catechins are contained in the palisade layer and polyphenol oxidase is found in the epidermal layer. Amino acids are contained in the vacuoles. The chemical composition of tea leaves is shown in Table 1. Among the components the most characteristic are the tea catechins. They not only influence the taste, but their content level also determines the type of tea that is produced. The total content of polyphenols in tea leaves is 25-30% on a dry weight basis and is mainly made up of flavanols. During the processing of black tea, about 90-95% of the flavanols undergo enzymatic oxidation to become products that are directly responsible for the characteristic color, astringency, and unique taste of tea brews. Another important component is amino acid. As shown in Figure 2, the concentration of amino acids is the highest in the first crop and gradually decreases in the second and the third crops. In addition, chlorophylls are present in tea flush and a high concentration of chlorophylls was reported to be associated with a grassy taste.

Fig. 1. The Internal Structure of a Tea Leaf (Schematic drawing)

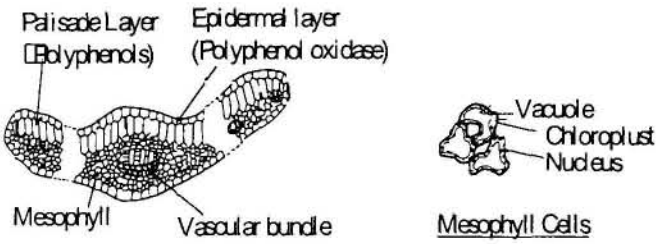
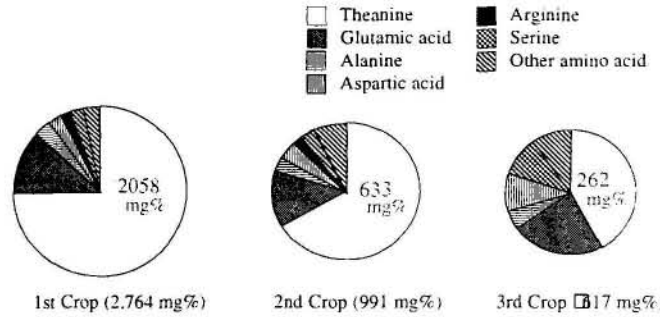


Figure 1. The Internal Structure of a Tea Leaf (Schematic drawing)

Table 1. Chemical composition of tea leaves

Constituent	% of Total Dry Wt.
Protein	15
Fiber	26
Pigment (chlorophylls, carotenoids, flavanoids)	2
Lipids	7
Caffeine	4
Polyphenols (catechins)	30
Amino Acids	4
Minerals	5

Figure 2. Total amino acid composition in fresh tea leaves of 1st, 2nd and 3rd crops



AROMA COMPOSITION OF BLACK TEA

The aroma of black tea is a mixture of numerous different volatile compounds. The main components are linalool, linalool oxides (*trans* and *cis* - furanoids and pyranoids), geraniol and 2-phenyl ethanol, which show floral note. C6-alcohols such as (*E*)-2-hexenol and (*Z*)-3-hexenol contribute to the fresh aroma.

Recently, the production of crush-tear-curl (CTC) tea is rapidly increasing with the rising popularity of tea bags throughout the world. The flavor of CTC tea is inferior to that of orthodox black tea because of the high level of carbonyl compounds and the low concentration of hexenyl esters, linalool, linalool oxides, and other desirable compounds. Due to their superior flavors, Keemun tea from China, Darjeeling tea from India and Uva tea from Sri Lanka are the three most famous black teas in the world. The aroma of Keemun tea has a rosy and woody note, while that of Uva tea contains a

sweet daphne flower-like fragrance with a refreshing green note. Darjeeling tea has a so-called Muscat-like aroma, accompanied by a somewhat attractive woody note. The aroma characteristics can be portrayed by gas chromatographic analyses as shown in Figure 3. The major differences in the aroma profiles are in the concentrations of linalool, linalool oxides, and geraniol.

AROMA FORMATION DURING MANUFACTURING OF BLACK TEA

Various aroma compounds of black tea develop during the four main stages of tea production, beginning with the fresh green leaf stage, the withering stage, fermenting stage after rolling, and the final product stage following firing. The total area of the pie chart represents the total yield of the aroma concentrate from the materials and each section shows the amount of individual main components. Hexenols, linalool and the oxides, methyl salicylate and geraniol increase remarkably during fermentation. During firing, a large amount of volatiles are lost and the total amount of aroma complex is reduced[1].

Fig. 3. Comparison of aroma profiles of various black teas

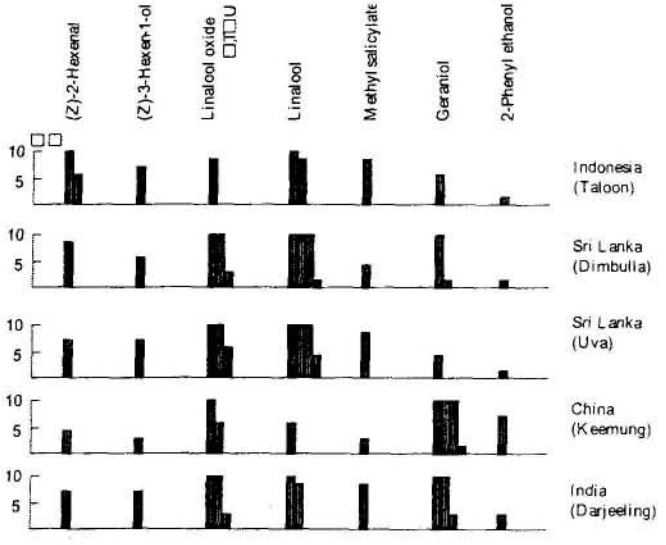
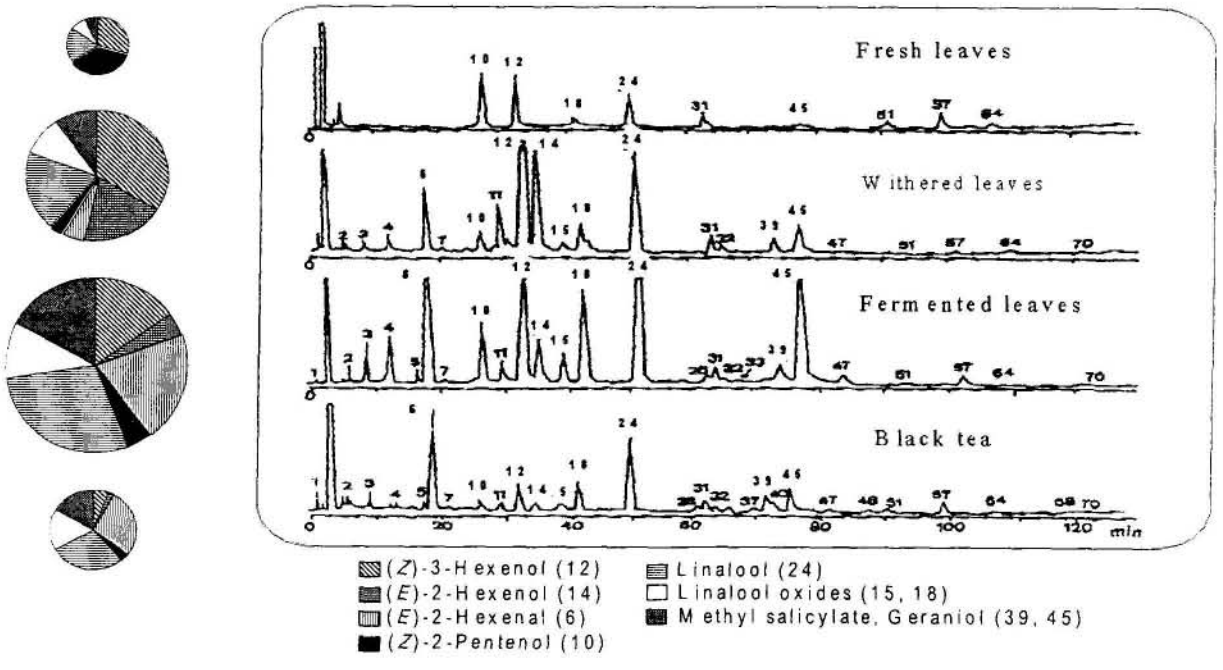


Fig. 4. Aroma formation during black tea manufacturing

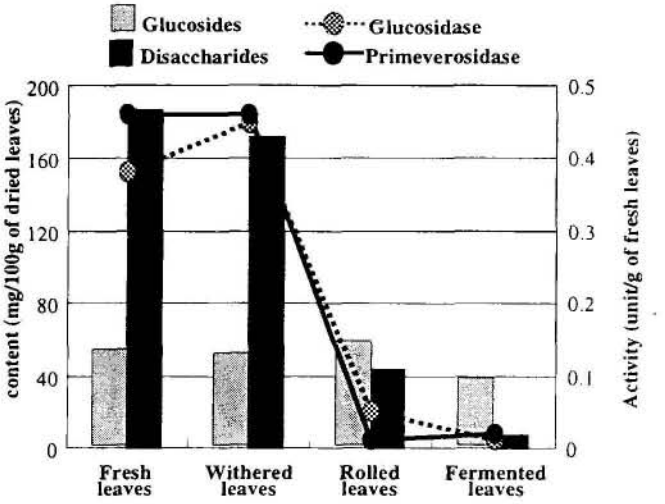


CHANGE IN THE CONTENT OF AROMA PRECURSOR GLYCOSIDES AND THE ACTIVITY OF GLYCOSIDASE DURING BLACK TEA MANUFACTURING

Recently, many glycosides, which contain main alcoholic aroma compounds of black tea in the form of aglycons, were isolated from fresh tea leaves. It is understood that aglycons are released by endogenous glycosidases during the manufacturing process of withering, rolling and fermentation. Moreover it is also known that the sugar moieties of the glycosides are mainly glucose - a monosaccharide, and primeverose - a disaccharide. Wang et al. investigated the change in the amount of the glycosides during the black tea manufacturing process and in the glycosidase activities of tea leaves by synthesizing main glycosides[2]. Fresh leaves contain more disaccharides than glucosides due to the high concentration of primeverosides. During the withering process, the amounts of both glucosides and primeverosides remained almost the same as those in the fresh leaves. During rolling, the primeverosides markedly decreased, whereas the level of glucosides was retained. After fermentation hardly any primeverosides remained, whereas the glucosides were still retained. Primeverosides are considered to be the main precursors of black tea aroma as described in the studies of Sakata's group[3]. Glycosidase is also deactivated after fermentation as shown in Figure 5. The tea leaves are mechanically destroyed during the rolling process, enabling the glycosidases to become more active and to increase the likelihood of interacting with the substrates. In addition, the alcoholic aroma compounds are mainly formed from the glycosides during the rolling process.

A detailed future study of the numerous enzymes present in tea leaves would undoubtedly contribute the elucidation of the mechanisms of the aroma formation during tea processing.

Fig. 5. Changes in glycoside content and glycosidase activity during black tea manufacturing process



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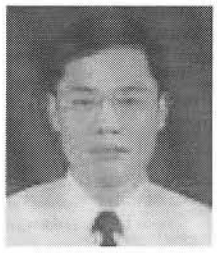
THE EXTRACTION AND PURIFICATION OF THEANINE

LIN Zhi*, TAN Jun-feng, LU Hai-peng, YANG Yong, YIN Jun-feng and CHEN Hao



Dr. Linzhi graduated from the Postgraduate School of the Chinese Academy of Agricultural Sciences in 1988. He has done a considerable amount of research on tea chemistry and on processing which is an essential link, at the Tea Research Institute. He received the Ministry of Agriculture Science prize in 1996, CAas Science prize in 2001 and the Zhejiang Province Science Prize in 2001. Despite a young age, he has published 25 research papers in the field of tea chemistry and tea processing. He studied for two years in Japan. There he engaged in the systematic study on the manufacture Sencha and Gabron tea. He has now been appointed Head of New Products Department of the Tea Research Institute in China where he is working on tea processing and new tea product developments. This is the future for tea. Dr. Linzhi worked on the extraction and purification of theanine. He has developed the processing techniques for three new green tea types. He has just completed three months exchange at the Unilever R & D Colworth.

ABSTRACT

Theanine is a unique amino acid in tea plant, which makes up 0.4%~3% of the solid matter in a tea shoot. It was found recently that theanine has many physiological activities and can be widely used in food and beverage industries as a new type of functional food ingredient. Theanine has become a new value added product followed the tea polyphenols in tea industry. To develop the extraction technique of theanine, the effects of several factors on extraction rate including volume of water, temperature and time of extraction have been investigated. The extraction conditions have been optimized by method of orthogonal design, then the optimal condition of extraction was determined and the purification of crude theanine was further researched. Results showed that the optimal condition of extraction was 1:20 (tealeaf/ water) at 90°C for 30 min. The product of theanine content >50% could be prepared by using 732 cation resin and WA-2 resin.

Keywords: China; extraction; purification, theanine

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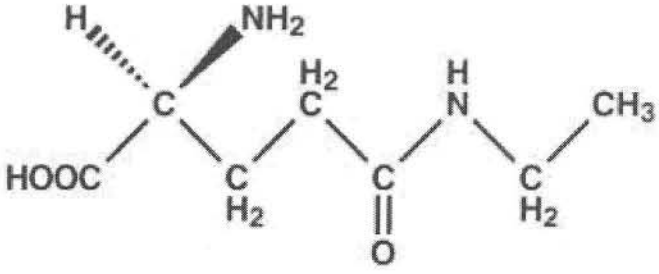
INTRODUCTION

Tea is one of most popular beverages in the world. It not only gives specific taste and flavor, but also has many physiological and functional effects due to such compounds as polyphenols, caffeine, amino acids, etc. [1-3] that it has. Theanine is a unique amino acid in tea plants, which enhances the taste of infused tea. It makes up 0.4%~3% of the solid matter and accounts for about 50% of all free amino acids in a tea shoot [4-5]. Theanine was first discovered by Sakato in 1950, and its chemical structure was determined to γ- ethylamino-L-glutamic acid [6] (Fig. 1).

Fig. 1. The chemical structure of theanine



Many studies have demonstrated that theanine can inhibit the excitation caused by caffeine, decrease

the blood pressure, cause a relaxation effect and prevent ischemic neuronal damage, etc. [3-4]. Its prevention of virus infection has also been reported recently [7]. Because of its good taste and favorable physiological effects on human, theanine could be widely used in food and beverage industries as a new type of functional food ingredient. Theanine has become a new value added product followed the tea polyphenols in Chinese tea industry [8]. To develop an effective extraction technique of theanine, the effects of several factors on extraction rate including volume of water, temperature and time of extraction had been investigated; then the optimal condition of extraction was determined and the purification of crude theanine was further researched.

MATERIALS AND METHODS

Materials

Leaf sample of roasted green tea was produced by the Wuyi Tangji Tea Company of Zhejiang Province, China. The sample was ground and sieved (60 meshes per inch). Theanine was purchased from Sigama Chemical Co. (St Louis, MO, USA) and amino acid standard H was purchased from Pierce Co. (Rockford, Illinois, USA). 732 cation resin and WA-2 resin were purchased from Hangzhou Shuanglin Chemicals Co. (Hangzhou, China). Other reagents were the highest grade commercial products.

Extraction and Purification of Theanine

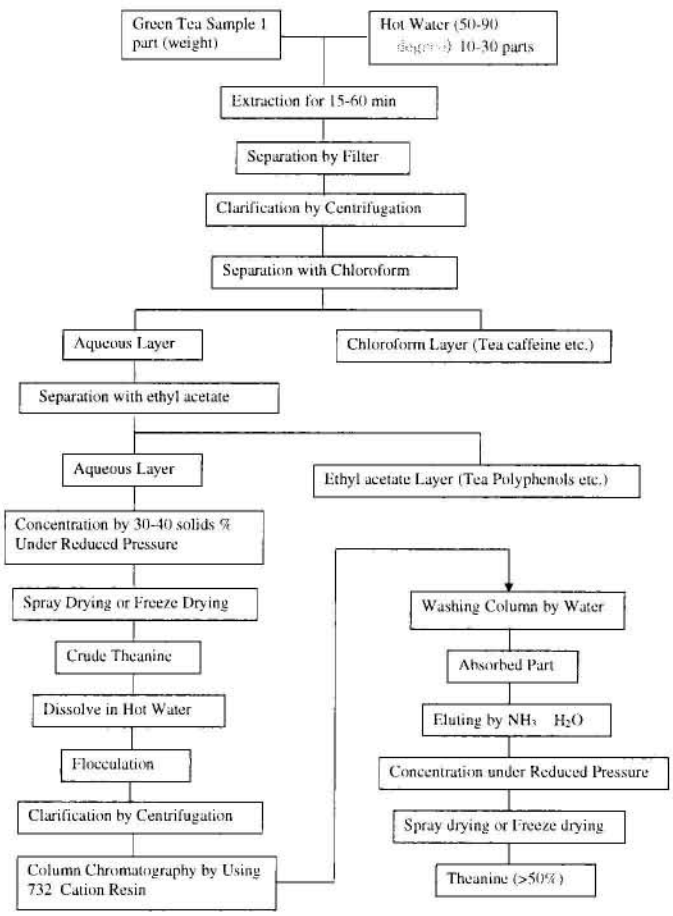
The method of extraction and purification of theanine followed is shown in Fig. 2.

HPLC Analysis of Theanine

The sample solution was filtered through a millipore filter (0.45μm). The filtrate (50 ul) was blended with 50 μl of 0.5M $NaHCO_3$, 50 μl of 1% DNFB, after a derivatization period of 1 hr at 60°C 100 μl of 0.01M KH_2PO_4 was added, and then analyzed by Waters-600 HPLC. The chromatographic conditions were as follows:

Injection volumes: 10μl
Column: 200 X 4.6 mm KF-AA 5μ
(Dalian Institute of Chemical Sciences Physics, CAS, China)
Column temperature: 30°C
Mobile phase: Solvent A: 60 mM NaAC buffer (pH 6.4)
Solvent B: Acetonitrile/H_2O (1:1, v:v)
Flow rate: 1.0 ml per min--
Controller: Waters 600 Controller
Detector: Waters 2487 Dual » Absorbance Detector
Gradient: Solvent A: solvent B (85:15) to solvent A: solvent B (0:100) by linear gradient during 30 min

Fig. 2. The method of extraction and purification of theanine



RESULTS AND ANALYSIS

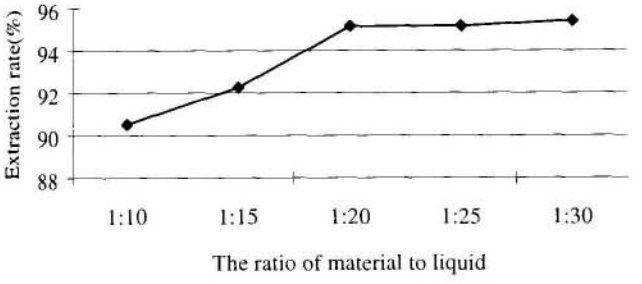
Extraction of Theanine

Effect of water volume: 5.0g of ground tea sample was placed with 50ml, 75ml, 100 ml, 125ml and 150ml boiling distilled water, respectively, and extracted for 30 min on a boiling water bath at 90°C(degrees). Effect of water volume on the extraction rate of theanine was investigated. Results in Table 1 and Fig. 3 showed that an increase in water volume from 1:10 to 1:20 increased the extraction rate of theanine. At the ratio of material to liquid higher than 1:20, the extraction rate of theanine almost remained the same (about 95%).

Table 1. The effect of water volume on the extraction rate

Water volume	Extraction rate1 (%)	Extraction rate2 (%)	Extraction rate3 (%)	Average
1:10	91.78	90.45	89.33	90.52
1:15	89.17	93.1	94.49	92.25
1:20	94.86	95.65	94.94	95.15
1:25	95.45	95.09	94.95	95.16
1:30	94.62	96.29	95.35	95.42

Fig. 3. The effect curve of the ratio of material to liquid (90°C, 30min)

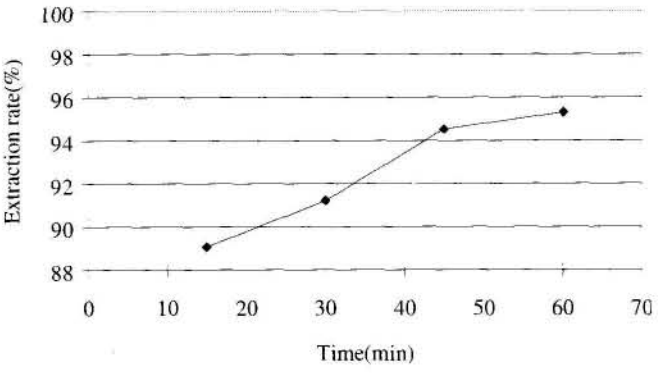


Effect of extraction time: 5.0g ground tea sample was placed with 75 ml boiling distilled water at the ratio of 1:15, and extracted on a boiling water bath at 90°C for 15 min, 30 min, 45 min, and 60min, respectively. Effect of extraction time on the extraction rate of theanine was investigated. As shown in Table 2 and Fig. 4, an increase of extraction time from 15 min to 40 min greatly increased the extraction rate of theanine. For extracted over 40 min, the extraction rate of theanine only got a slight increase.

Table 2. The effect of extraction time on the extraction rate

Time (min)	Extraction rate 1(%)	Extraction rate 2(%)	Extraction rate 3(%)	Average
15	89.63	89.04	88.53	89.07
30	89.17	93.10	91.38	91.22
45	95.15	94.55	93.92	94.54
60	95.64	94.46	95.84	95.32
75	97.97	97.74	97.78	97.83

Fig. 4. The effect curve of extraction time (1:15, 90°C).

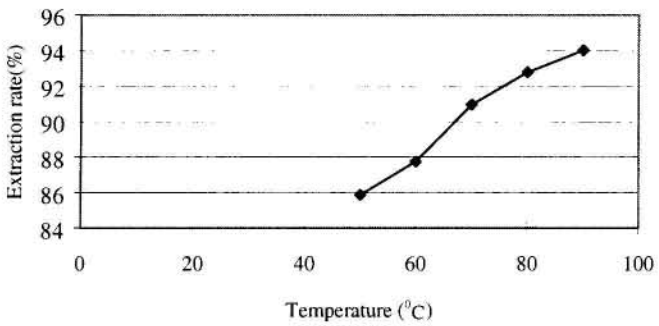


Effect of extraction temperature: 5.0 g ground tea sample was placed with 75 ml boiling distilled water at the ratio of 1:15, and extracted for 30 min on a boiling water bath at 50°C, 60°C, 70°C, 80°C, 90°C, respectively. Effect of extraction rate of theanine was investigated. As shown in Table 3 and Fig. 5, the extraction rate of theanine increased continuously with the increase of extraction temperature.

Table 3. The effect of extraction temperature on extraction rate.

Temp-erature(°C)	Extraction rate 1(%)	Extraction rate 2(%)	Extraction rate 3(%)	Average
90	94.38	93.39	94.26	94.01
80	93.74	92.25	92.31	92.76
70	90.93	90.72	91.20	90.95
60	87.46	87.76	88.02	87.75
50	85.57	86.49	85.57	85.88

Fig. 5. The effect curve of extraction temperature (1:15, 30min)



Determination of Optimal Condition of Extraction

On the basis of the above experimental results, the orthogonal design was applied to optimize the extraction conditions of theanine. Three factors and their levels were selected as showed in Table 4.

Table 4. The factor and level in test

Levels	Water volume (A)	Temperature (B)	Time (C)
1	1:10	70°C	15min
2	1:15	80°C	30min
3	1:20	90°C	45min

Table 5. The result of orthogonal design

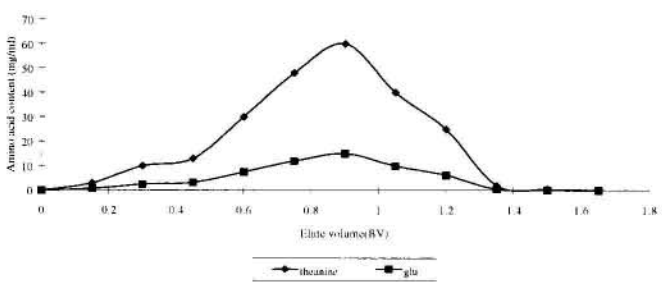
No.	Water volume (A)	Temperature (B)	Time (C)	Extraction rate (%)
1	1:10	70	15	90.142
2	1:10	80	30	95.838
3	1:10	90	45	96.603
4	1:15	70	30	92.063
5	1:15	80	45	93.325
6	1:15	90	15	97.179
7	1:20	70	45	93.572
8	1:20	80	15	95.489
9	1:20	90	30	97.906
k_1	282.584	275.778	282.811	
k_2	282.568	284.653	285.807	
k_3	286.968	291.689	283.501	
k_1'	94.195	91.926	94.270	
k_2'	94.189	94.884	95.269	
k_3'	95.656	97.229	94.500	
R	1.466	5.304	0.999	

By the F test and R analysis, results showed that effect of three factors, water volume (A), temperature (B) and extraction time (C), on the extraction rate of theanine was in a sequence as follows: B>A>C. The optimal condition of extraction was $A_3B_3C_2$, indicated 1:20, 90°C and 30min (Table 5).

Purification of Theanine

Purification by using 732 caion resin: The H^+ formed 732 cation resin was equilibrated with pH 3.0 citric acid buffer solution, then put into a column (20×200 mm). The crude theanine extract (10 g) was dissolved in boiling de-ionized water (100 ml), and the solution pH was adjusted to 3.0-3.5. The solution was flocculated and centrifuged, the supernatant was injected into the column. After washed by water, the column was eluted with 1.5 mol/L $NH_3 \cdot H_2O$. The fraction of eluent was collected, and the theanine content in each fraction was analyzed by HPLC. The elute curve showed that theanine was eluted from the resin together with glutamic acid (Fig. 6). 1.5 BV eluent of $NH_3 \cdot H_2O$ was collected and concentrated under reduced pressure at 60°C, and then freeze-dried. The theanine content of freeze-dried powder was 27.5%.

Fig. 6. The elute curve of theanine from 732 cation resin



Purification by using WA-2 resin: WA-2 resin was equilibrated with pH 6.5 buffer solution, then put into a column (20×200mm). The crude theanine (0.4g) purified by 732 cation resin was dissolved in

de-ionized water (100ml) and the solution pH was adjusted to 6.5. The solution was injected into the column, and then the column was eluted with pH 6.5 buffer. The elute curve of theanine was illustrated in Fig. 7. The eluent of 1.5BV was collected and freeze dried. The theanine content of freeze dried powder was 51.5% by HPLC analysis (Fig. 8).

Fig. 7. The elute curve of theanine from WA-2 resin

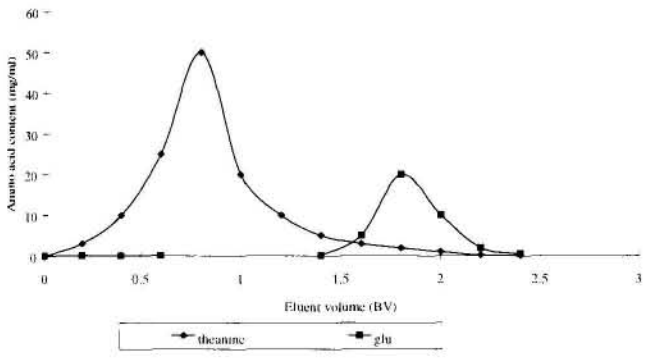
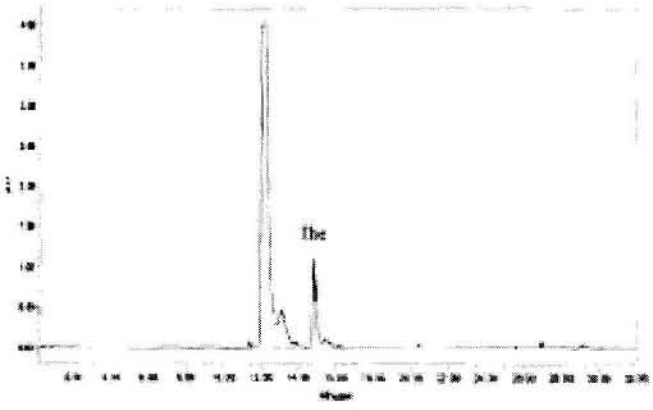


Fig. 8 HPLC chromatogram of theanine of the sample prepared by the resin



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Chairman's Comments

Thank you very much Dr. Linzhi for informing us of the range and quantities of tea products which are produced in China. This is a surprise for many of us here, who do not know much about the work going on in China on value addition products from tea.

Chapter 27

NEW HORIZONS OF VALUE ADDITION FOR ASSAM TEA

Mridul Hazarika*

Mridul Hazarika is a tea bio-chemist. He is presently the Director of Tea Research Association of India. He joined Tea Research Association in 1978 and during that time till now has held various positions in the institute. He has to his name about 40 publications in tea and he has worked extensively on tea aroma and Darjeeling flavour particularly, and highlighted the importance of terpenoids for the unique Darjeeling flavour. Since then he has established the relationship of different components of thearubigins and their relationship to quality that is an extremely complex area. Currently he is coordinating a number of all India tea projects.

ABSTRACT

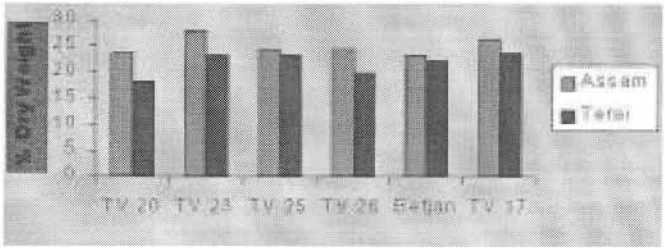
Value addition of tea is an important requirement under present market scenario of tea. With the increase in demand for RTD, tea extract for various usages has made this area of study more attractive. Assam tea, gifted with high level of some chemical constituents, can be best utilized for this purpose.

What are the characteristics of Assam tea? How these chemical constituents contribute to various taste perceptions is what most of us know. My previous speakers have spoken at length on the crisis faced by the tea industry and the possible ways to overcome this crisis. Available knowledge on the chemistry of tea needs to be utilized to make the tea more remunerative. How best the properties of Assam tea can be exploited is what is going to be focused in the subsequent part.

While exploiting the properties of Assam tea, reference is being made here to *Camellia sinensis* (var. *assamica*) that is rich in some chemical constituents. This variety is grown not only in Assam, it is also grown in large areas in Dooars, Terai, Assam Valley and Cachar valley where we refer to all these teas as Assam tea. There is another variety called *Camellia sinensis* variety *sinensis* that is mostly grown in Darjeeling district. I shall not be discussing in detail about this tea. In this presentation I am referring to only variety *assamica* that has certain rich attributes to be used to our advantage.

Assam tea is rich in catechins, lutein, theanine and volatile flavoury constituents. Catechin content of Assam variety is normally as high as 28-30%, which in certain cases was found to be 32%. Regional variation of catechins was also found to be remarkable (Fig. 1).

Fig. 1. Regional variation of catechin



The effort made in last five decades was towards productivity -- higher production. Thrust now is directed on quality. Cost reduction and improvement of quality need to proceed simultaneously for making tea more remunerative. Our sole objective now is directed towards quality. Brief highlights of issues before Assam tea are presented in Fig. 2.

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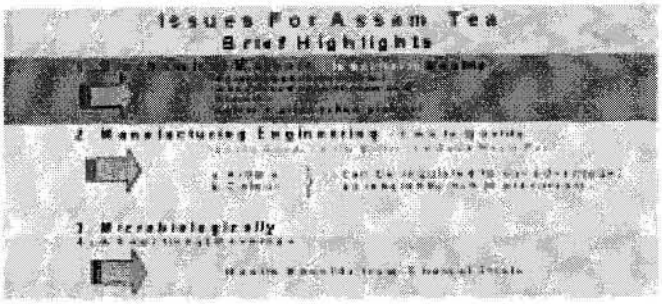
Fig. 2. Issues for Assam Tea



Strategies for achieving better quality of Assam tea involve special package of field practices to induce formation of high level of catechins. In addition to catechins, it is possible to induce large number of other chemical constituents, through the field practices, to be used to our advantage. Tea, as a drink or as a functional beverage, could make use of this approach for enhancement of the level of chemical constituents in tea plant. Tables 1-3 show the chemical constituents of an average tea.

Table 1. Chemical constituents in black tea

Chemical Component	% Dry Wt.
Flavanols	1-3
Flavanol and their glycosides	2-3
Theaflavins (TF)	1-2
Thearubigins (TR)	10-20
Phenolic Acids & Depsides	4-5
Pigments	0.5 - 0.6
Polysacchrides	1 - 22
Proteins	15 - 17
Caffeine	3 - 4
Amino Acids	5 - 6
Sugar	3 - 4
Organic Acids	0.5 - 0.6
Mineral Substances	4 - 5
Volatile Substances	0.02 - 0.03

With fall in prices, we have two options to look for. One is better quality to be acceptable to consumer as a beverage and the other is the functional use of tea. Previous speakers have elaborated at length on large number of products based on tea. Quality is the consumer's choice that is variable and the concept of quality is highly dynamic. It is difficult to predict the consumer's perception.

Table 2. Some major black tea chemical components present in a cup of tea

Black Tea Component	% Dry Wt.
Flavanols	0.03 - 0.09
Flavanol and glycosides	0.06 - 0.09
Theaflavins	0.03-0.06
Thearubigins	0.3 - 0.6
Amino Acids	0.05 - 0.18
Caffeine	0.09 - 0.135
Sugar	0.09 - 0.12
Aroma	Trace
Organic Acids	0.015
Mineral Substances	0.09 - 0.12
Protein	Trace

Table 3. Vitamin in black tea

Vitamin	µg/100g leaf	µg/cup	% daily requirement from 5 cups
Thiamine	135.0	2.3	0.8
Riboflavin	1266.0	21.5	5.0 - 7.0
Niacin	7500.0	127.5	5.2
Folic Acid	76.0	1.3	6.0 - 13.0
Pantothenic Acid	1260.0	21.4	1.4 - 6.0
Biotin	82.5	1.4	3.1

There are a large number of precursors that have functional properties, and the products derived from many of these precursors have also important functional properties. Imbalance of chemical constituents by inducing higher accumulation of a particular component may affect the acceptability of tea as a common beverage. Black tea contains some important minerals, adding value to a cup of tea. Table 4 shows minerals received from intake of 5-6 cups of tea.

Table 4. Mineral provided by black tea

Mineral	Daily intake from 5-6 cups of tea (mg)	% Daily requirement from 5-6 cups
Potassium	9.82	25
Manganese	1.80	45
Magnesium	17.50	5
Copper	0.17	7
Zinc	2.40	10
Sodium	5.40	1

Tocklai has made significant progress in improvement of quality of average tea of high yielding planting material through control of process conditions. Brightness of high yielding planting materials, such as TV19, 22, 23, 25 and 26, could be increased 30-40% by regulating processing conditions, particularly withering.

Quality determining biochemical constituents, such as TF and lower molecular weight TR, are increased considerably to produce the requisite brightness if processing parameters are properly regulated. Tables 5 and 6 show the TF and low molecular TR contents in control and experimental tea.

Table 5. Comparison of TF (% Dry weight) in experimental tea with control

	W 1	W 2
TV 1	1.80	1.40
TV 23	2.17	1.54
TV 25	1.62	1.34
TV 26	1.95	1.63

W1 - Experimental

WR - Control

Table 6. Comparison of low molecular weight TR in experimental with control

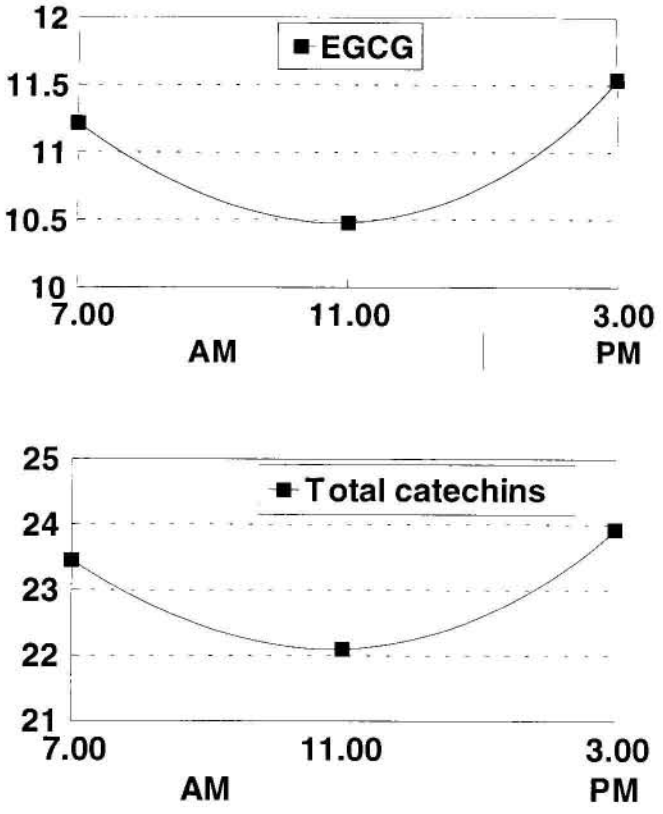
	W 1	W 2
TV 1	6.84	3.88
TV 23	6.65	5.43
TV 25	5.64	3.67
TV 26	6.99	5.29

W1 - Experimental

WR - Control

Precursor-product relationship is very important. Ideal precursor balance can lead to a quality product or a product of choice for various functional uses. Significantly diurnal variation has also been observed in relation to catechins, as shown in Fig. 3, which influences quality of tea plucked at various times during the day.

Fig. 3. Diurnal variation of catechins



Isolation of these important functional components by convention has been going on for quite sometime. Recent efforts on Supercritical Fluid Extraction (SCFE) to obtain food grade products would be highly useful. Figs. 4 and 5 show the flow sheet with condition of extraction, and the comparison of some of the extracts obtained from conventional chemical method and SCFE in our own experiments.

Fig. 4. Comparison of some components in chemical & SCFE methods

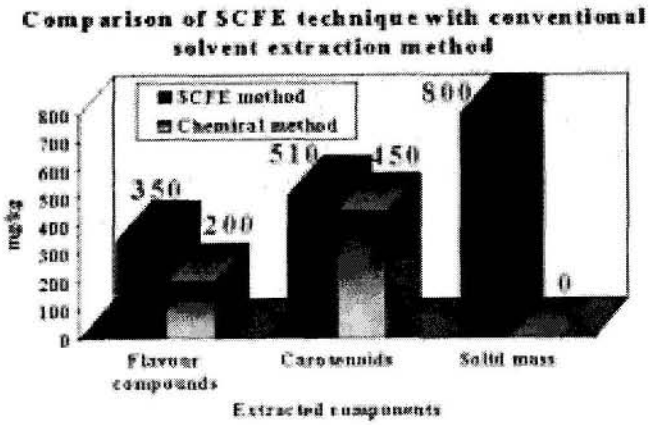
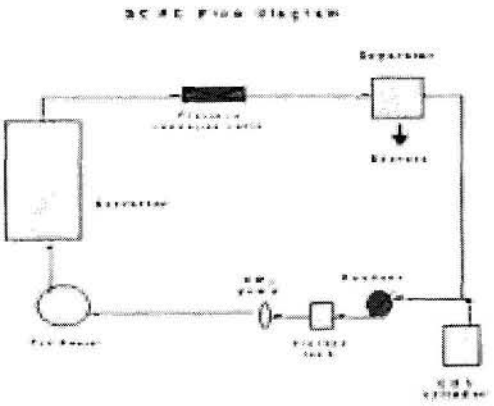


Fig. 5. Flow Sheet of SCFE



SCFE Extraction of Tea sample

CO_2 flow rate = 45-50 kg/hr

(a) at 40°C for 1 hr at 100 bar yielded 40 ml of flavour components

(b) at 40°C for 1 hr at 120 bar yielded 10 ml of flavour with carotenoid components

(c) at 40°C for 1 hr at 200 bar yielded 30 ml carotenoid components

Chapter 28

IMPROVEMENT OF FLAVOUR QUALITY OF CTC BLACK TEA BY GLYCOSIDASES IN TEA LEAVES

Kanzo Sakata*, Masaharu Mizutani, Seun-Jing Ma, and Wenfei Guo#

Educated at Kyoto University, Dr. Kanzo Sakata is originally a Natural Products Chemist. His interest is in the isolation and determination of the structure of bioactive natural products. He became interested in tea chemistry while working with a Chinese scientist at Shizuoka University. Particularly his interest is the chemical basis of the floral tea aroma found in Oolong tea. Oolong tea is manufactured by very special process and is one of the least understood teas in biochemical terms. After moving to Kyoto University, he clarified the gene encoding β-Primeverosidase His recent research interests have focused the role of β Primeverosidase in tea plants as well as the utilization of specific glycosidase to improve the flavour for tea quality.

ABSTRACT

We have been interested in molecular basis of floral tea aroma formation during so-called fermented tea, especially oolong tea. We have isolated aroma precursors of floral tea aroma such as linalool, geraniol ß-primeverosides. This fact stimulated us to identify the glycosidase responsible for the floral aroma formation and to clone the gene of the enzyme. Studies on the substrate specificity of the enzyme showed that ß-primeverosidase is a diglycosidase (a disaccharide-glycoside specific glycosidase), which is very specific to ß-primeveroside and shows very low ß-glucosidase activity.

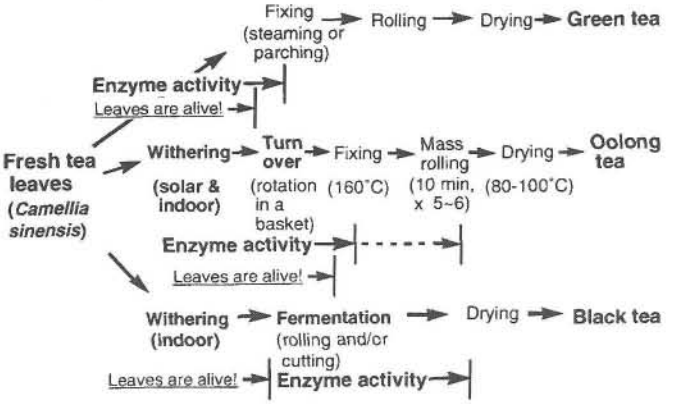
We also clarified that benzaldehyde is generated from a cyanohydrin ß-glucoside in tea leaves indicating that ß-glucosidases also play some important roles in tea aroma formation.

Based on these research results together with published data related to tea aroma, we would like to show a few proposals to improve the quality of CTC black tea flavor.

INTRODUCTION

Basically juvenile fresh leaves of tea plants (*Camellia sinensis*) can be processed to green tea, oolong tea or black tea *via* different processing procedures as shown in Fig. 1.

Fig. 1. Outline of basic tea manufacturing processes



In green tea production tea leaves are steamed or pan-fired to kill endogenous enzymes after being plucked. However, endogenous enzyme reactions occur to result in floral tea aroma formation as well

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as coloring in so-called "fermented tea (oolong tea and black tea)" production. The most important differences between the two types of fermented teas are how the endogenous enzymes react. In the case of black tea manufacturing, especially CTC black tea production, plucked juvenile tea shoots are cut, teared and curled into fine pieces to well mix the enzymes with substrates, resulting in production of pigments such as theaflavins, thearubigins, *etc.* and flavors such as green note and floral aroma. Too much production of green note is becoming one of big problems lowering its quality in CTC black tea production. Here tea leaves are not alive anymore. On the other hand tea leaves are let to be alive for much longer time until they are heated for fixing during the oolong tea manufacturing (Fig. 1), although tea leaves are left under serious stresses such as water deficiency, injuries, *etc.* A lot of beautiful floral aroma is known to be generated during the processing. This is the very important point to be pointed out.

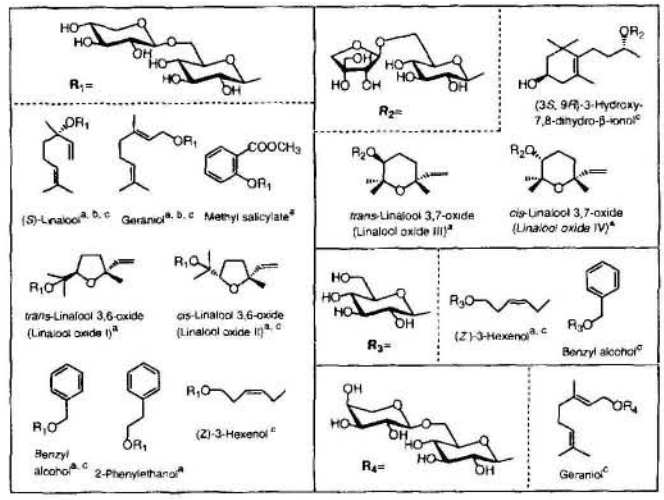
Based on our research results of molecular mechanism of the floral aroma formation in oolong tea and black tea, we would like to propose a method to produce new type of black tea which can be produced as easily as CTC black tea and with much more floral aroma.

ISOLATION AND IDENTIFICATION OF AROMA PRECURSORS OF THE FLORAL TEA AROMA

Takeo *et al.* carried out a very interesting experiment on aroma analysis of different types of tea, steamed green tea, perched green tea, and oolong tea, which are manufactured from the same tea leaf sample of cv. Benihomare (1). Much more volatile compounds were generated during the oolong tea manufacturing process. Especially enormous amount of geraniol is produced compared with the other two types of green teas. Most of these major volatiles are known to contribute to the floral tea aroma (2). We were interested in the molecular mechanisms of the floral tea aroma formation.

First we tried to isolate and identify aroma precursors of these floral tea aroma, geraniol, linalool, benzyl alcohol, *etc.* We have succeeded in identification of most of these major floral tea aroma precursors (3,4). Figure 2 summarizes all of the alcoholic aroma precursors isolated so far from tea leaves (cvs. Shuixian and Maoxie) for oolong tea production and those (cv. Yabukita) for green tea by us and Prof. Kobayashi's group of Ochanomizu University (5). We found that these entire almost alcoholic aroma compounds are present as ß-primeverosides in fresh leaves for oolong tea and only linalool oxides III and IV were present as 6-*O*-ß-D-apiofuranosyl-ß-D-glucopyranosides. Kobayashi's group isolated geranyl ß-vicianoside as a new aroma precursor (6).

Fig. 2. Aroma precursors isolated from tea leaves.



[a]*from oolong tea leaves (cv Maoxie);*
[b]*from oolong tea leaves (cv Shuixian);* [c]*from green tea leaves (cv Yabukita)*

Most of the important floral tea aroma precursors have been shown to be mainly present as ß-primeverosides in oolong tea leaves (Fig. 2). These facts strongly suggest that some specific glycosidase(s) should be concerned with the floral tea aroma formation.

PURIFICATION OF ß-PRIMEVEROSIDASES FROM TEA CULTIVARS FOR GREEN TEA, OOLONG TEA AND BLACK TEA PRODUCTION AND ITS SUBSTRATE SPECIFICITY

We have purified primeverosidases from fresh leaves of three different species of tea plants for black tea (var. *assamica*), oolong tea (cv. Shuishan) and green tea (cv. Yabukita) production. Enzymic characteristics of these ß-primeverosidases are summarized in Table I (3). We can conclude that these primeverosidases are enzymaticaly identical, although they showed slight differences in TOF-MS analysis and peptide mapping.

Table 1. Enymatic characteristics of the ß-primeverrosidases from fresh tea leaves

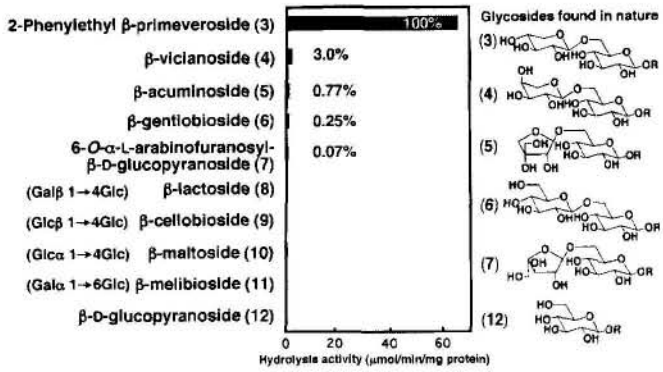
	ß-primeverrosidase		
	Black Tea[a]	Oolong Tea[b]	Green Tea[c]
M.W. (TOFMS)	60,300	60,200	60,500
pI	9.5	9.5	9.4
Optimum temp. (°C)	45	45	45
Stable temp. (°C)	40	40	45
Optimum pH	4	4	4
pH stability	4-5	3-5	4-5
Specific activity (unit/mg)	0.99	0.98	0.90

[a]*Camellia sinensis* var. *assamica*; [b]*C. s.* var. *sinensis* cv. Shuixian; [c]*C. s.* var. *sinensis* cv. Yabukita

We obtained many kinds of synthetic or natural disaccharide glycosides of 2-phenylethanol and examined substrate specificity of the ß-primeverosidase from cv. Yabukita with these substrates (Fig. 3) (7). The ß-primeverosidase showed very high selectivity towards ß-primeveroside, although it hydrolyzed other natural disaccharide glycosides with 1-6 glycosidic linkage, but not any unnatural synthetic ones. To our surprise, the ß-primeverosidase was not able to hydrolyze ß-D-glucopyranoside.

Now we can understand that many kinds of floral tea aroma such as geraniol, linalool, benzyl alcohol, *etc.* are stored as disaccharide glycosides such as ß-primeverosides in tea leaves and generated during tea processing by the action of endogenous glycosidases, mainly ß-primeverosidase.

Fig. 3. Substrate specificity of ß-primeverosidase from tea leaves (cv. Yabukita)



Substrates: 2-phenylethyl diglycosides (10 mM); enzyme: ß-primeverosidase (0.22 unit/ml) purified from tea leaves; hydrolysis activity was calculated after incubation in 20 mM citrate buffer (pH 6.0) at 37°C. The amount of liberated 2-phenylethanol was analyzed by HPLC (ODS-AQ, 33% MeCN) analysis.

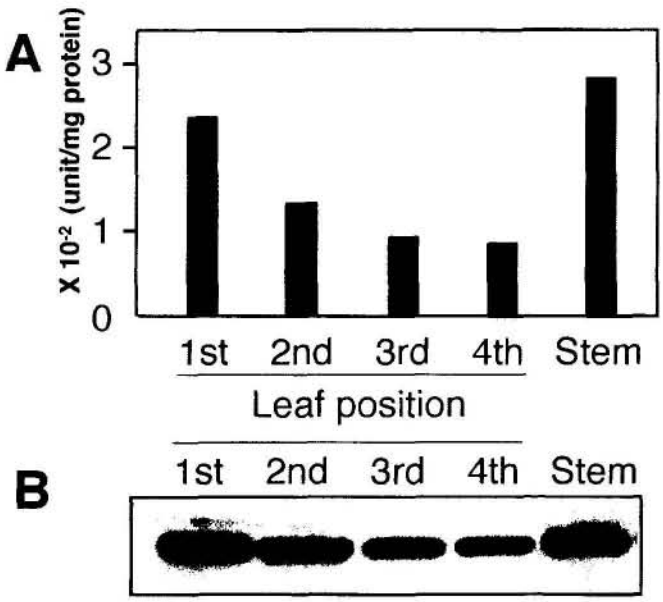
MOLECULAR BIOLOGICAL STUDIES ON THE TEA LEAF ß-PRIMEVEROSIDASE AND ITS DISTRIBUTION IN TEA LEAVES

The cDNA encoding the ß-primeverosidase was cloned in the conventional manner (8). P-sort analysis predicted that a signal peptide of 28 amino acid residues is present, indicating that the mature protein is secreted outside the cells. These five asparagine residues are the putative N-glycosylation sites, and the β-primeverosidase is a glycoprotein. The cDNA was overexpressed in *E. coli* and confirmed ß-primeverosidase activity.

ß-Primeverosidase protein with His-tag was used to prepare anti-ß-primeverosidase polyclonal antibody using a rabbit (8). The antibody was found to have high sensitivity as well as high selectivity. This tool is quite useful for quantification of ß-primeverosidase in various tea samples and for screening of ß-primeverosidase from other plants.

ß-Primeverosidase activity of each part of tea shoot of cv. Yabukita was measured with *p*NP ß-primeveroside (8). The higher activity was observed in the more juvenile leaves (Fig. 4A). Fairly high activity was observed in stem, suggesting the enzyme is transported. The results of Western blotting experiment (Fig. 4B) with the polyclonal antibody are in good accordance with the ß-primeverosidase activity profile. These results indicate that the younger leave contain more ß-primeverosidase.

Fig. 4. Distribution of the ß-primeverosidase in tea shoots



A : Measurement of ß-primeverosidase activity;
B:Immunoblot analysis with the anti-primeverosidase antibody

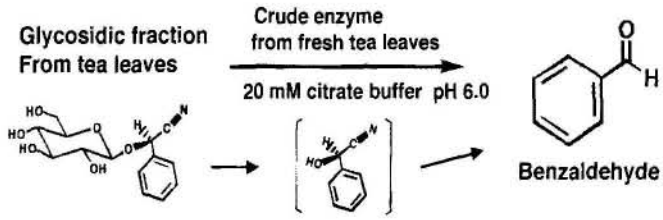
Previously we measured indirectly the amounts of glycosidic aroma precursors and glycosidase activity in each part of tea shoots (9). The younger leaves contain the more glycosidic aroma precursors and the higher glycosidase activities. These results are very similar to those obtained by the b-primeverosidase activity measurements (Fig. 4A) and the Western blotting experiment (Fig. 4B).

The polyclonal antibody was also used to know intercellular localization of the ß-primeverosidase in tea leaves. ß-primeverosidase was observed to be localized in cell wall and cavity area among cells (8). Aroma precursors such as ß-primeverosides are present in vacuoles. They are never encountered in ordinary conditions but stresses such as insect feeding, infection by microbes and wounding let them react to release bioactive substances such as monoterpene alcohols, *etc.* (10). These compounds are tea aroma themselves. So the aroma formation in tea leaves during manufacturing oolong tea is concluded to be the result of defense responses of tea leaves against various stresses.

ß-GLUCOSIDASE INVOLVED IN TEA AROMA FORMATION

Benzaldehyde is also an important aroma in made tea, especially in oolong tea and black tea (Fig. 5).

Fig. 5. Benzaldehyde formation from prunasin in tea leaves and benzaldehyde in various kinds of made teas



Benzaldehyde contents in various kinds of made teas

Green tea	Longjing tea	Oolong tea	Black tea	Dark tea
0.2	0.1	1.1	1.1	0.5%*

*The rate against all aromatic components, calculated by GC peak area.

We were interested in the molecular mechanism of the aroma formation and isolated prunasin as an aroma precursor of benzaldehyde from juvenile leaves of cv. Yabukita (11). Benzaldehyde is considered to be generated during tea processing as shown in Fig. 5 in the same manner as in cherry (12). This process accompanies by generation of

toxic HCN and is also considered to be one of defense mechanisms of plants.

PLUCKING TIMING AND TEA AROMA FORMATION

Generally speaking tea leaves for green tea and black tea manufacturing are plucked just after a tea shoot with the third-leaf. However, they wait until the forth-leaf or the fifth-leaf is developed and plucked a tea shoot with the third- or the forth-leaf when they produce oolong tea. It sounds quite interesting to us from tea aroma formation points of view. We obtained two types of black tea made by the same method from each of different tea leaf materials (shoots 1 and 2) shown in Table 2. of a tea cultivar for oolong tea production in Fujian (China). These two kinds of black tea were analyzed by GC (Table 2.)(unpublished data).

Table 2. Volatile constituents of black tea made from tea leaves in different maturity

Compounds	Shoot 1[a]	Shoot 2[b]
3-Pentenol	36.5[c]	114.5
(Z)-2-Pentenol	26.0	42.1
(Z)-3-Hexenol	14.8	23.9
trans-Linalool 3,6-oxide	61.3	297.1
cis-Linalool 3,6-oxide	72.0	309.1
Benzaldehyde	29.9	100.4
Linalool	77.0	119.4
3,7-Dimethyl-1,1, 5,7-octatriene-3-ol	54.8	288.4
α -Terpineol	15.2	34.7
trans-Linalool 3,7-oxide	13.4	73.5
cis-Linalool 3,7-oxide + Methyl salicylate	22.7	79.2
Hexanoic acid	23.5	145.0
Geraniol	139.0	105.8
Benzyl alcohol	10.0	39.0
2-Phenlethanol	28.1	49.1
Benzyl cyanide + ß-Ionone	50.9	39.6
(Z)-Jasmone	6.8	0.0
Nerolidol	15.0	36.0
Jasmine lactone	10.9	15.0
Indole	7.3	11.5

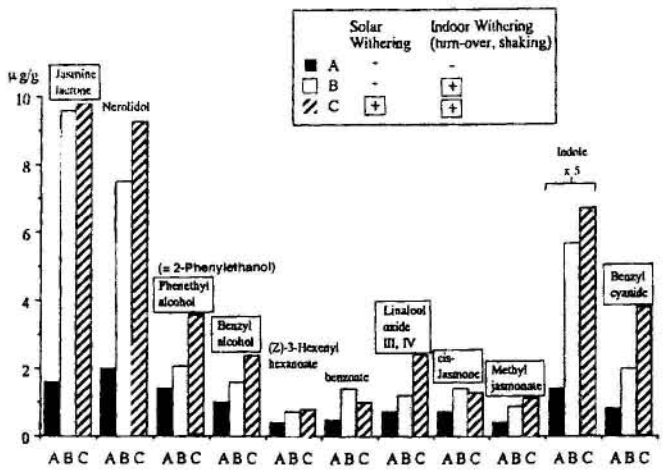
[a] *Tea shoots with up to 3rd leaves;*
[b] *Tea leaves 1st~3rd leaves plucked from those with the 4th or 5th leaves;*
[c] *μg/dry weight 20 g.*

Almost all volatile compounds observed were liberated much more from the black tea made from the material tea leaves plucked at the timing for oolong tea production. This fact suggests that plucking timing for oolong tea production should be worthwhile to introduce in the new type black tea production to improve its quality of tea aroma, although some volatile compounds may be undesirable for tea aroma. We will be able to find the best timing for it after several trials.

DEVELOPMENT OF TEA AROMA COMPOUNDS DURING WITHERING PROCESSES FOR POUCHONG TEA

Pouchong tea is a new type of oolong tea mainly made in Taiwan, which is prepared *via* very light fermentation process. The tea infusion is likely to be that of green tea and rich of floral aroma. Yamanishi reported effectiveness of withering processes (solar and indoor) in floral tea aroma formation (Fig. 6) (2). Much more aroma compounds such as jasmine lactone, 2-phenylethanol, benzyl alcohol, indole, *etc.*, are generated from the tea leaves after withering processes comparing with those from tea leaves without withering. During withering processes tea leaves are alive and subjected to stresses of water deficiency, indicating that aroma formation in tea leaves is a response of tea leaves against stresses. We can understand that generation of the floral tea aroma is a result of responses of juvenile tea leaves against stresses.

Fig. 6. Development of tea aroma compounds during withering processes for Pouchong tea



PROPOSALS FOR PRODUCTION OF A NEW TYPE OF BLACK TEA AS EASILY PRODUCED AS CTC BLACK TEA AND WITH RICHER AROMA THAN CTC BLACK TEA

CTC black tea is known to contain too much green note due to leaf alcohol [(*E*)-2-hexenal] and much less amount of aroma compounds such as linalool, linalool oxide, methyl salicylate, *etc.* (2). It is important to increase the amount of floral aroma to improve the flavor quality of CTC black tea. The basic experimental results shown above are quite informative to produce a new type of black tea by modifying the conventional CTC black tea production method. We would like to sum up several important points for this purpose.

1) Use material tea leaves of the juvenile shoots with upto the 4th- or 5th-leaves.
2) Before processing of so called fermentation, material tea leaves should be subjected to more stresses (not only withering but also slightly injuring of tea leaves) as in the case of oolong tea production.
3) Temperature control during the processing may be effective
4) Glycosidases such as diglycosidases, which can effectively hydrolyze the tea aroma precursors shown above and are now commercially available from Amano Enzyme Co. Ltd. in Japan, may be applied to obtain better flavor quality.
5) Too fine cutting of tea leaves may cause enormous production of the green note and is not necessary for the floral aroma formation from glycosidic aroma precursors

We do hope this paper will give some inspirations to any reader who are interested in improving the flavor quality of the conventional CTC black tea and manufacturing a new type of black tea that can be made as simply as CTC black tea and with richer floral aroma than CTC black tea.

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Concluding Remarks of the Chairman

We are impressed by the painstaking, fundamental work that the biochemists undertake in order that one day we shall be able to apply that work in the factories to improve the quality of our tea.

Chapter 29

STUDY TO PROMOTE THE INDUSTRIAL EXPLOITATION OF GREEN TEA POLY PHENOLS IN INDIA

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ICS-UNIDO & University Institute of Pharmaceutical Sciences,
Panjab University, Chandigarh

ABSTRACT

A study to analyse the content of epigallocatechin gallate in Indian cultivars with aim to identify the best cultivar was sponsored by International Centre for Science and High Technology of UNIDO. The study was accomplished at University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh. It was aimed to benefit the Indian tea industry to develop value added products for emerging market of non-beverage tea products for populations not accustomed to drinking green tea but seeking green tea benefits.

The study developed the HPLC analytical procedure for estimation of EGCG and analysed a wide variety of cultivars from different geographic regions in India to identify the varieties rich in EGCG content. Experiments were also performed optimize extraction conditions for selection of solvent, sample solvent ration, time, temperature and particle size. The EGCG content of a large number of Indian cultivars will be presented. The study also estimated ECG content of these samples.

Full text of the paper has not been received till the time of printing ; Editors

Chapter 30

PANEL DISCUSSION ON VALUE ADDITION

Chairman K.V. Raghavan : Secretary M. Hazarika

Members empanelled for discussion:

1. Professor Tei Yamanishi: Pioneer investigator of tea flavor
2. Dr. Kanzo Sakata; Developed technology to alter grassy to floral note
3. Dr. N. Muraleedharan; Expert on pesticide residues on tea quality
4. Dr. S. Ramaswamy: Tea manufacture & machinery expert
5. Dr. S. Ravindranath; Tea chemist — holds several patents
6. Mr. Prafull Goradia; Contemporary Targett Tea Brokers
7. Mr. Radhey Dixit: Senior most Tea planter from Darjeeling
8. Mr. Sanjay Kapur; Specialty tea specialist
9. M. Piyush Desai; Chairman of Gujrat Tea Traders & Packers Association,
10. Mr. Bharat Sarronwala; Senior tea executive
11. Dr. F. Rahman, Tea Advisory & Training Service, Siliguri
12. Dr K.V. Raghavan Director IICT Hyderabad ***Chairman***
13. Mr. Paul Choudhury, Chairman of Mohurgong Gulma Tea Company
14. Dr Pawan Kapur, Designer of controlled withering trough
15. Dr. Rajindra Singh, Vice President Nestle R&D in Singapore
16. Mr. Krishna Kumar: Tata Tea executive, now M.D. Tata Hotels
17. Dr. Mridul Hazarika, Director Tocklai ***Secretary***
18. Scientist deputed by NCL, Pune
19. Chemist deputed by TRI, Srilanka
20. Scientist deputed by TRF, Kenya
21. Dr. Lin Zhi Tea Research Institute Hangzhou, China
22. Dr. Karan Vasisht C/o UNIDO, Trieste, Italy

SUMMARY BY M. HAZARIKA

Dr. K. V. Raghavan, Chairman of Panel Session, opening the discussion, mentioned that diversification of tea application is going to be an important area of research in view of the high content of industrially useful components in the tea. He emphasized the need to identify, isolate and test these components for assessing their application potential in high value pharma and speciality chemicals. He then invited Dr. Tei Yamanishi of Japan to initiate the discussion with Japanese experience. Tei Yamanishi said that 25

years of research experience in Japan has established the benefits of drinking tea with high catechin content. Several scientists from various parts of the world confirmed these findings. Japanese scientists are also exploring new application areas for tea based products.

Mr. Lin Zhi, from China in his opening remarks, stated that setting up of commercial plants to produce high value tea products continues to be an area of great commercial interest globally. Scientists from China have already established commercial plants based on polyphenols (200 lbs produced in China), polysaccharides and others from tea. Their experience shows that value addition to tea may be possible by:

1. Developing special tea grades with high content of specific functional components by suitably altering methods of processing.
2. By making more varieties of natural tea available for drinking to increase the consumption.
3. By extracting various components from tea, such as catechins and colouring materials and develop exclusive formulations based on them for commercial applications.

Dr. K. Vashisht spoke about the importance of EGCG as an anti cancer agent and stressed the need to identify high EGCG containing tea varieties. He also highlighted the need to isolate caffeine for higher end pharamecutials and marketing of caffeine and decaffeinated tea varieties as two distinct consumer products. He cautioned that it would not be easy to isolate caffeine and make formulations due to its pH sensitivity. Marketing of acetylated catechins can be tried in view of its favourable metabolism within human body.

Dr. M. Hazarika in his address highlighted the need to study digallates, including theaflavin digallate as important tea downstream products. He also emphasized that product evaluation should be done scientifically and a strong information base should be created to promote product diversification.

Assam tea is rich in chemical constituents with therapeutic properties. Potentiality of each component need to be evaluated and separation technology to be developed to make Assam tea more attractive commercially. He further stated that flavoring agents from Darjeeling and Assam tea have good prospects to be developed as food additives.

Dr. Rahman brought to the notice of participants regarding the availability of green dust tea in two grades, viz. dust and yellow leaves which have no market value. These can be used as food additives by taking advantage of its fibre value. He mentioned that good prospects exist for tea based consumer products like tooth paste and mouth wash.

Mr. P. Ramakrishna from Tata Tea, responding to the panelists view, informed about the manufacture of polyphenols in different concentrations by two Indian companies. Decaffeinated tea produced in India is cheap as compared to that from other countries. He stated that supercritical extraction (SCE) is a cost effective technique for high value and low volume products. He emphasized that collaboration of various institutions is essential for development of downstream product industry based on tea. Funds earmarked for such studies by Tea Board, NABARD and NTRF wouyld be of great value for pursuing commercially relevant R&D in this area.

Dr. Jaswant Singh (RRL Jammu), highlighted the need to provide quality assurance certificate to major tea consignments to ensure that Indian tea is free of pesticidal and microbial loads and high level of stability and authentic chemical signature.

Use of chemical markets for quality assurance is also important. For diversification into high value tea products, EGCG need to be given highest priority. Theaflavins are more potent than Vitamin E as antioxidant. Thianines, on the other hand, get degraded to glutamins in the body system. This can boost up the immune system in human beings against growing +ve and –ve bacteria. The small theanine molecule can provide significant value addition to the product. He appealed to the Tea Board to allocate different assignments to research institutions considering their expertise and facilities.

Dr. Sharmah, NABARD, Mumbai stated that bankers greatly depend upon scientists for evolving technologies. The NABARD is financing development/projects through NTRF. It has evolved schemes for the scientists to receive funds directly for their research projects which can contribute to socio-economic development.

Dr. N. K. Jain spoke about the downward trend in international tea prices and the immediate need for introducing high value products in international and national markets. At present, tea production is higher than its consumption. Hence diversification is the need of the hour.

Concluding the discussion, Dr. Raghavan, the Chairman highlighted the following to make the research on diversification more need based and meaningful:

1. A wide range of tea byproducts can be made commercially with varying levels of market value.
2. Conversion of tea downstream products into commercially attractive pharmaceutical and other speciality compounds need sustained research efforts.
3. The currently available information on antioxidants and anticancer compounds from tea is rather scanty and hence there is scope for further work.
4. Tea extraction process should be developed as per the specifications of downstream products. They should undergo performance evaluation, both in-vitro and in-vivo. Multi-stage extraction, leading to more than 95% extraction efficiency may be needed in some cases. Counter current and SCFE can also be tried.
5. Fractionation will be needed to get minimum number of constituents with maximum activity. Sophisticated techniques may be required to achieve sharper cuts. Chromatographic fractionation of multi component systems requires special equipments.
6. It is necessary to isolate the chemical constituents from potential fractions and subject them to user tests.
7. Association of the user industries is very essential for success of product development.
8. TRA and Tea Board should adopt a co-coordinated approach involving CSIR, ICMR and other national laboratories, where the facilities of converting natural products to commercial and medicinal products are already available.
9. Information network has to be strong in tea downstream product area. Pre-feasibility information and status of various technologies should be made available to potential users.
10. Market potentiality should be assessed by competent agencies.
11. Sponsors for the tea downstream product/ process development programme should be identified to formulate suitable R&D projects.

Dr. K. V. Raghavan Chairman, Panel Session opening the discussion mentioned that diversification of tea is going to be an important area of research in view of high content of functional components. He emphasized on identifying appropriate functional components and their application. He then requested Dr. Tei Yamanishi to initiate the discussion to be followed by other members of the panel.

Tei Yamanishi said that 25 years of experience in Japan observed the benefits of tea drinking due to high catechin content present. These findings were confirmed by many other scientists in the world.

Mr. Lin Zhi from China stated that setting up of commercial plants to produce high value products of profit continue to be an important problem. Scientists from China have came out with products based on polyphenols (200 lbs produced in China), polysaccharides etc. from tea. Increase of the value of tea may be possible by :

- Making tea with high content of functional components by changing methods of processing.
- By making available more kinds of tea for drinking to increase the consumption.
- By extracting various components from tea, such as catechins, coloury material etc.

Dr. K. Vasisht spoke on importance of EGCG as cancer preventing component and emphasized on identifying high EGCG varieties. He also highlighted on isolation of caffeine and marketing of caffeine and decaffeinated tea as two separate products.

He cautioned that it would not be easy to isolate caffeine and make formulations due to pH sensitivity.

Marketing of acetylated catechins can be tried as the conversion within the body could take place.

Dr. M. Hazarika highlighted the need of digallates including theaflavin digallate. He also emphasized on product evaluation and said that information network should be adequate for an integrated need based approach in product diversification.

Assam tea is rich in chemicals which are very important from the therapeutic point of view. Potentiality of each component can be evaluated and be separated to make tea more remunerative.

He further stated that Tea flavors from Darjeeling and Assam Tea have high prospect as food additive.

Dr. Rahman had spoken about Dust Green Tea where two grades viz. dust and yellow leaves have no market value. If these can be used as tea powder for food additives to have fibre value. He also mentioned about tea based products like tooth paste, mouth wash etc.

Dr. P. Ramakrishna from Tata responding to the panelists view spoken about the availability of polyphenol in different concentrations. He mentioned about two firms manufacturing these in India. Decaffeinated tea produced in India is cheap as compared to other countries. He emphasized the need of Super Critical Food Extractor (SCFE) as a cost effective technique. Collaboration of various institutions is essential, he further emphasized. Funds allotted for these studies by Tea Board, NABARD, NTRF would be of great help in this direction, he stated.

Dr. Jaswant Singh (RRL Jammu) highlighted the need of providing quality assurance certificate mentioning that it was free of pesticides, microbial load, stability and chemical signature. Use chemical markers for quality assurance is also important he mentioned.

Diversification or high value addition to tea products. EGCG is an wonderful molecule. Theaflavin are

more potent even 4 times than that of Vitamin E as antioxidant. Thianines on the other hand, the smallest component in tea. In body system it get degraded to glutamins. This can boost up the immune system in human being against growing +ve and –ve bacterias. The small molecule (theanine) can give a great value addition to the product.

There are areas to be looked into in case of pharmaceutical and therapeutical requirement. He appealed to the Tea Board for different assignments to various institutions.

Dr. Sharma, NABARD, Bombay participating in the discussion stated that Bankers bank upon the scientists for cost effective funding which should be accepted by all. NABARD is financing through NTRF. NABARD have schemes for the scientists to fund directly for research projects which would help in the socio-economic development.

Dr. N. K. Jain spoke about the price of tea going down and simultaneous demand for high value product. The production being higher than the consumption which is responsible for the problem. Hence diversification is the need of the hour.

Concluding the discussion Dr. Raghavan highlighted the following to make the research on diversification more effective and meaningful :

1. We have a range of tea byproducts with different values.
2. Conversion of downstream products to pharmaceutical and other bioactive compounds is necessary.
3. Scanty information on antioxidants and anticancer activity is available and hence scope for further work.
4. Extraction process should be tailored for the given requirements. Then extracts should go for performance evaluation, both in-vitro and in-vivo.
5. Fractionation will be needed to get minimum numbers of constituents with maximum activity.
6. It is necessary to isolate the chemical constituents from potential fractions.
7. Association of the user industries is very essential.
8. Integrating chemical and pharmaceutical industries should be explored.
9. Multistage extraction leading to 95% extraction is acceptable. Counter current and SCFE should be attempted.
10. Chromatographic fractionation of multiple components with SEPBOX (cost 2 crore) will be useful.
11. TRA, Tea Board should have a co-coordinated approach with CSIR laboratories where the facilities of converting natural products to commercial and medicinal products are available.
12. Information network should be strong. Pre-feasibility report, status of various technologies should be available.
13. Potentiality of markets etc. should be identified.
14. Sponsors for the programme should be identified.

Concluding comments by Dr. N.K. Jain Executive Organising Secretary , 3rd International Conference of Global Advances in Tea Science

Dr. Raghavan, to me it has been a journey of discovery. Dr. Ramakrishna of the house of Tatas and Dr. Sharma of the Unilever pointed out that we have information on the subject of value added products from tea in India, which is not widely known. Tthe scientists of our neighbors Japan and China have developed commercially viable technologies to manufacture high value products from low-grade tea or waste products. I request Dr. Raghavan and Dr. Hazarika to prepare a special report for the International Journal of Tea Science

on the subject of value addition. We shall host information on the ISTS website about:

- Technologies available for value added products from tea
- Mechanism for effective transfer of technologies
- Working system of arranging buyer-seller linkages as the ultimate objective.

A special project should be prepared about manufacture of high-value product/s selling for $ 60 /kilo or so. Such a project can be put up, say, in Himachal, which has tripled its productivity of high quality tea since 1984. Due to lack of buyers, the situation today is so grim that 70% of Himachal tea bushes remain unplucked while three of the public sector factories have closed down. I suggest a team led by Dr. Vasisht, Dr. Raghavan, Dr. Ramakrishna to consult scientists of Japan & China, t and prepare a feasibility report for manufacture of Value added products in Himachal. The objective would be to help interested manufacturer/s to obtain technology, supported by funds from NABARD with the help of Dr. MR Sharma, which can also be availed for field development with technical support from the CSIR scientists. I trust that the team members of this Panel will accept this challenge. I commend this project to the attention of the State Government of Himachal Pradesh who sent a very strong team of tea planters and IHBT for nominating CSIR tea scientists to attend this conference.

**** For live discussion of the panel on Value Addition, listen to the attached audio CD***