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Nutritional and medicinal improvement of black tea by yeast fermentation

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Abstract

Black tea fermentation with *Dabaryomyces hansenii* for 10 days resulted in accumulation of major vitamins, such as A, B_1, B_2, B_{12} and C in sufficient quantities to fulfil the recommended dietary allowances (RDA). Fermentation of tea by yeast resulted in reduction of caffeine and excess tannins in significant amounts. After fermentation, the amount of theophylline was increased to make fermented tea a potent bronchodilator. Tea fermentation with *D. hansenii* improved its nutritional and medicinal values. Our observations suggest that intake of fermented tea is advantageous over black tea in terms of its nutritive and therapeutic values.

Keywords: *Dabaryomyces hansenii*; Fermented tea; Theophylline; Riboflavin; Ascorbic acid; Tannins

1. Introduction

Tea is an aqueous infusion of dried leaves of the plant *Camellia sinensis* (L.) O. Kuntze, and is the most popular beverage, consumed by human society world-wide and second only to water. Tea is a dietary source of antioxidant nutrients, such as carotenoids, tocopherols, ascorbic acid and non-nutrient phytochemicals generally classified as flavonoids and also regarded as safe by the US food and drug administration (Wu Christine & Wei, 2002). It is reported that tea extracts inhibit human salivary amylase (Zhang & Kashket, 1998) and disturb digestion and nutrition. Numerous studies have demonstrated that aqueous extracts of black tea and green tea posses anti-mutagenic, anti-inflammatory, hypocholesterimic, anti-diabetic, anti-bacterial (Hamilton-Miller, 1995), anti-tumor (Mitscher, Jung, & Shankel, 1997; Yoshizawa et al., 1987), anti-UV induced oxidative DNA damage (Wei et al., 1999) and anti-cariogenic (Wu Christine & Wei, 2002; Yoshizawa et al., 1987; You, 1993) activities in a variety of experimental animal systems. Excess consumption of tea is also harmful as it causes mortality and coronary morbidity whereas coffee shows the opposite (Woodward & Tunstall-Pedoe, 1999). A major component of tea, caffeine, causes impairment of the mechanical properties of growing bone in early life (Mitsuhiro et al., 2002). Polyphenols of tea are shown to be inhibitors of the absorption of non-heme iron (Disler, Lynch, & Charlton, 1975; Derman, Sayers, Lynch, Hrlton, & Rothwell, 1977). In recent years, the general population has demonstrated increased awareness and interest in "functional foods", i.e. foods with positive health benefits or bioregulatory functions. Researchers have explored not only desirable food habits but also beverages that may contribute to overall health and disease prevention (Wu Christine & Wei, 2002). It is difficult to implement a new type of diet, even with positive effects and so better to modify the currently available one with little or no change. The aim of the present study was to modify the most-consumed beverage tea by yeast fermentation to increase benefits in terms of nutritional enrichment of vitamins and medicinal improvement by decrease in toxic components (at high levels of consumption) and increase in physiologically important components.

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2. Materials and methods

2.1. Yeast culture

Debaryomyces hansenii MTCC 1073, yeast culture obtained from IMTECH Chandigarh, India, was maintained on malt yeast agar medium slants (MTCC medium No. 6) and preserved at 2–8 °C temperature. Actively growing yeast cells grown in above medium slants were used for inoculation into tea extract.

2.2. Preparation of fermented tea

Orange Pico type (Assam tea company India) tea leaves (2 g), and sucrose (2 g) were added to 100 ml of boiling water and steeped for 15 min without loss of steam. The infusion was cooled to room temperature and then filtered. The resulting clear filtrate was similar to the tea beverage consumed as black tea. This filtrate was taken in 250 ml Erlenmeyer flasks and sterilized at 15 Psi for 15 min. After cooling to room temperature flasks were inoculated with two loops full of actively growing yeast, *D. hansenii*, cells from slant culture. Flasks were incubated at 25 °C in an orbital shaker incubator at 150 rpm. After 10 days of incubation, the tea ferment was centrifuged and supernatant was taken for analysis. Unfermented (uninoculated) tea was also incubated simultaneously, as above, and centrifuged supernatant was taken for analysis.

2.3. Analysis

The fermented and unfermented tea extracts were analyzed for various vitamins and medicinally important physiological active components. Vitamins, such as β-carotene, thiamine, riboflavin and ascorbic acid were analyzed by AOAC methods (Cunnif, 1995); niacin and cyanocobalamin were analyzed according to the Indian Pharmacopoeia (1996).

Riboflavin content was also analyzed by microbiological assay (AOAC 94033), using *Lactobacillus casei* sub sp. *rhamnosus* MTCC 1408. Theophylline and caffeine were analyzed by HPLC using water:acetonitrile:acetic acid (940:60:10) as mobile phase, UV–visible detector at 280 nm,1 ml/min flow rate, using a single pump SHIMADZU HPLC system and C18 column at room temperature. Total tannins were analyzed by the Folin and Denial method of AOAC (Cunnif, 1995).

3. Results

3.1. Yeast fermentation

Tea fermentation by *D. hansenii*, using as mono-culture was carried out. Growth of yeast was found on the second day of fermentation which was detected by increased OD at 600 nm. Increase in growth continued up to the third day and no growth was found after the fourth day. Little remaining glucose was found on the third day of fermentation and traces on the fourth and nil on subsequent days of fermentation. After the fourth day a gradual decrease in total tannins and caffeine and increase in vitamins and theophylline were noted. On the tenth day of fermentation, maximum amounts of vitamins were produced and they were reduced on subsequent days of fermentation. No more reduction of tannins or caffeine were noted after the tenth day; therefore, fermentation for ten days was optimized (Fig. 1).

Yeast fermentation of tea extract resulted in formation of nutritionally important vitamins, such as riboflavin, β-carotene, thiamine and ascorbic acid (Table 1). Improvement of medicinal, physiologically active components in fermented tea was found by increased theophylline content, decreased caffeine and total tannins

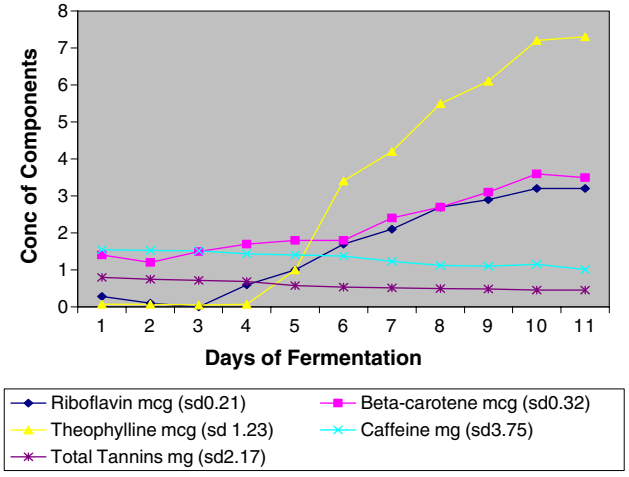

Fig. 1. Yeast fermentation of black tea concentrations are per ml. Series: (1) riboflavin; (2) β-carotene; (3) theophylline; (4) caffeine; (5) total tannins.

Table 1
Nutritional improvement of vitamins in fermented tea using *D. hansenii*

Vitamin	Concentration/ml			
	Black tea ($n = 9$) (μg)	SD	Fermented tea ($n = 9$) (μg)	SD
β-Carotene	1.4	0.033	3.6	0.055
Riboflavin	0.28	0.018	3.2	0.026
Thiamine	0.085	0.002	3.2	0.036
Cyanocobalamine	0.0	0.0	0.5	0.039
Ascorbic Acid	4.4	0.061	13	0.052

The values given above are averages of three experiments, each in triplicate, conducted on different occasions ($n =$ total number of samples).

Table 2
Medicinal improvement of physiological active components in fermented tea using *D. hansenii*

Component	Concentration/ml			
	Black tea ($n = 9$)	SD	Fermented tea ($n = 9$)	SD
Total tannins	0.8 mg	0.018	0.45 mg	0.007
Caffeine	1550 μg	1.870	1015 μg	1.224
Theophylline	0.07 μg	0.001	7.2 μg	0.030

The values given above are averages of three experiments, each in triplicate, conducted on different occasions ($n =$ total number of samples).

(Table 2). Experiments were done thrice, each time in triplicate and maximum CV was 5%.

4. Discussion

The fermentation method adopted to modify the value of tea in the present study, was easy to perform with household utensils. Tea has high levels of tannins, phenolic compounds, which form strong complexes with proteins and other polymers (Goldstein & Swain, 1963). These polyphenols inhibit the digestive enzymes by precipitation (Jolyn & Jolyn, 1964a) and also strongly inhibit the absorption of non-heme iron (Brune, Rossander, & Halberg, 1989; Derman et al., 1977; Disler et al., 1975; Halberg & Rossander, 1982). Tannins are important in food because of their astringency (Jolyn & Jolyn, 1964a), a contracting or drying taste (Mooncrieff, 1951). *Debaryomyces* yeast is known to utilize aromatic compounds when the fermentation is weak (Karasevich, 1978). *D. hansenii*, MTCC 1073, is known for riboflavin production (IMTECH, 2000). Various populations consume different fermented teas in which micro organisms, as a consortium, are used for fermentation (Chen & Liu, 2000). These fermented teas contain many yeasts of which a few may be facultative pathogens which infect immunosuppressed persons (Mayser, Fromme, Leitzmann, & Grunder, 1995). The consortium gives smell and taste which may not be liked by some people. To avoid the consortium culture, changing the smell and taste, *D. hansenii* was used as a monoculture for the present tea fermentation. The importance of tea for its nutritional (Wu Christine & Wei, 2002) and medicinal values (Hamilton-Miller, 1995; Mitscher et al., 1997; Wei et al., 1999; Wu Christine & Wei, 2002; Yoshizawa et al., 1987; You, 1993) and its harmful effects on health (Hattori, Kusumoto, & Namba, 1990; Mitsuhiro et al., 2002; Sehenker, 2001; Woodward & Tunstall-Pedoe, 1999; Zhang & Kashket, 1998) is well documented. In general, a person drinks 2 (Graham, 1978) to 4 cups (Weidner & Istvan, 1985) of tea per day (1 cup = 200 ml) (Halberg & Hulthen, 2000). If an average of 3 cups (600 ml) of fermented tea is taken daily, its nutritional improvement is progressive.

Daily intake of fermented tea provides 2160 μg of β-carotene which is better than black tea (840 μg) (Table 1) and takes care of the recommended dietary index of 2400 μg to an adult human (NNMB, 1995). Studies have shown that vitamin A and riboflavin play important roles in iron metabolism. The provision of these nutrients, along with iron, significantly improved iron nutrition and reduced anemia in young children and pregnant women when compared with those who received only one of the nutrients (Allen & Casterline-Sabel, 2001). Riboflavin deficiency is quite common in many parts of the developing world and clinical signs of angular stomatitis have been reported in several surveys. However, it has received little attention (Allen & Casterline-Sabel, 2001; Ramakrishnan, 2002). Daily intake of fermented tea provides 1.92 mg of riboflavin (Table 1) which is better than black tea (0.168 mg) and meets the recommended dietary index of 1.4 mg for an adult human (NNMB, 1995). Niacin is also an important vitamin in the human diet (Miller, 2003) and there was no difference between niacin contents in the tea extracts before and after fermentation (results not shown).

Generally, the thiamin recommended dietary index is met by food, but inhibitory effects of tea components on thiamin utilization have been reported (Wang et al., 1991). Daily intake of fermented tea provides 1.92 mg of thiamin (Table 1) which is better than black tea (0.051 mg) and meets the recommended dietary index of 1.2 mg for an adult human (NNMB, 1995). Cyanocobalamin deficiency is common in a country like India, where animal-based food intakes are low (DeMaeyar & Adiels-Tegman, 1985). Daily intake of fermented tea provides 0.3 mg of cyanocobalamin (Table 1) which is better than black tea (0.0 mg) and meets a part of the recommended dietary index of 1.0 mg for an adult human (NNMB, 1995).

Ascorbic acid (vitamin C) is an essential component of the human diet. Moreover vitamin C enhances iron absorption (Cook & Redd, 2001; Halberg & Hulthen, 2000) and is important in preventing megaloblastic anemia of infants (Jacob, 1994) and also reduces stomach cancer (Hemila & Herman, 1995). Daily intake of fermented tea provides 7.8 mg of ascorbic acid (vitamin C) (Table 1) which is better than black tea (2.64 mg) and meets a part of the recommended dietary allowance of 40 mg for an adult human (NNMB, 1995), but may be positive to health as it is synergistic with other vitamins and minerals. The effect of ascorbic acid on the inhibition of iron absorption by tannins was reported (Siegenberg, Baynes, & Bothwell, 1991; Tuntawiroon, Sritongkul, & Brune, 1991). Iron binding polyphenols are widespread in foods and in many beverages, such as wine, coffee and tea (Brune et al., 1989; Harborne, 1980). In fermented tea decreased tannins and increased

ascorbic acid concentrations are significantly useful for iron absorption and improved digestion. Black tea contains 0.8 mg/ml of total tannins which equals 160 mg per cup and 480 mg daily intake by tea alone. Fermented tea contains 0.45 mg/ml total tannins and gives 90 mg per cup and 200 mg as daily intake, which is sufficient for astringency taste.

Black tea has high concentrations of caffeine (1550 μg/ml). It is practically impossible to avoid caffeine as it is present in various foods, beverages and over the counter medications (Mitsuhiro et al., 2002). On balance, it is better to reduce the daily intake of caffeine. There was a decrease in caffeine content in fermented tea. A cup of black tea contains 310 mg caffeine and its daily intake, through tea, is 930 mg (when 3 cups are taken), whereas fermented tea contains 200 mg caffeine per cup and its daily intake will be 600 mg (Table 2).

Theophylline is a xanthine bronchodilator. It also causes neural excitability by binding to γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the cortex (Nardone, Buffone, Move, Lochner, & Tezzon, 2004). It is less rapidly absorbed when given orally but rapidly and widely distributed in the tissue after absorption. Its treatment dose, as a bronchodilator, is 0.18–1.0 g daily (Motaff, 1992). A cup of black tea contains 0.014 mg of theophylline (Table 2) and its daily intake is 0.042 mg, whereas fermented tea contains 1.44 mg theophylline per cup and its daily intake will be 4.32 mg, which is helpful, as it increases neurostimulation and additionally can become part of a therapeutic dose for patients on theophylline treatment.

Our observations suggest that intake of fermented tea is superior to black tea in terms of its nutritive and therapeutic value as it also did not show much change in taste and color after fermentation. This may be recommended for consumption as a modified beverage with higher nutritive values.

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References

Allen, L., & Casterline-Sabel, J. (2001). Prevalence and causes of nutritional anemia. In U. Ramakrishnan (Ed.), *Nutritional anemias* (pp. 7–21). Boca Raton, FL: CRC Press.

Brune, M., Rossander, L., & Halberg, L. (1989). Iron absorption and phenolic compounds: Importance of different phenolic structures. *European Journal of Clinical Nutrition, 49*, 140–144.

Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology, 5*, 834–839.

Cook, J. D., & Redd, M. B. (2001). Effect of ascorbic acid intake on newborn iron absorption from a complete diet. *American Journal Clinical Nutrition, 73*, 93–98.

Cunnif, P. (1995). *Official methods of analysis of AOAC* (16th ed., Vol. II). AOAC International suite 400 USA.

DeMaeyar, E., & Adiels-Tegman, M. (1985). The prevalence of anemia in the world. *World Health Statistics Q, 38*, 302–316.

Derman, D., Sayers, M., Lynch, S. R., Hrlton, R. W., & Rothwell, T. H. (1977). Iron absorption from a cereal-based meal containing cane sugar fortified with ascorbic acid. *British Journal of Nutrition, 38*, 261–269.

Disler, P. B., Lynch, S. R., & Charlton, R. W. (1975). The effect of tea on iron absorption. *Gut, 16*, 193–200.

Goldstein, J. L., & Swain, T. (1963). Changes in tannins in ripening fruits. *Phytochemistry, 2*, 371–383.

Graham, D. M. (1978). Caffeine-its identity, dietary sources, intake and biological effects. *Nutritional Reviews, 36*, 97–102.

Halberg, L., & Rossander, L. (1982). Effect of different drinks on the absorption of non-heme iron from composite meals. *Human Applied Nutrition, 36A*, 116–123.

Halberg, L., & Hulthen, L. (2000). Prediction of dietary iron absorption: An algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition., 71*, 1147–1160.

Hamilton-Miller, J. M. (1995). Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrobial Agents of Chemotherapy., 39*, 2375.

Harborne, J. B. (1980). Plant phenolics. *Encyclopedia of Plant Physiology, 8*, 329–402.

Hemila, H., & Herman, Z. (1995). Vitamins C and the common cold: A retrospective analysis of chalmers review. *Journal of the American College of Nutrition, 14*(2), 116–123.

Hattori, M., Kusumoto, T. T., & Namba, T. (1990). Effect of tea polyphenols on glucan synthesis by glycosyltransferases of *Streptococcus mutans*. *Chemical and Pharmaceutical Bulletin, 38*, 717.

IMTECH. (2000). In *Microbial type culture collection; catalogue of strains* (p. 121). Chandigarh, India: Institute of Microbial Technology.

Indian Pharmacopoeia. (1996). *Govt. of India, Ministry of health and family welfare* (Vol. I & II), Delhi: The Controller of Publications.

Jacob, R. A. (1994). Vitamin C. In M. E. Shils, J. A. Olson, & M. Shike (Eds.), *Modern nutrition in health and disease* (8th ed., pp. 432–448). Philadephia, PA, USA: Lea and Febiger.

Jolyn, M. A., & Jolyn, L. (1964a). Astrigency of fruits and fruit products in relation to phenolic content. *Advances in Food Research, 13*, 179–217.

Karasevich, I. (1978). Utilization of aromatic compounds by yeasts of genus *Debaryomyces*. *Mikrobiologiia, 47*(6), 985–991.

Mayser, P., Fromme, S., Leitzmann, C., & Grunder, K. (1995). The yeast spectrum of the tea fungus Kombucha. *Mycoses, 38*(7–8), 289–295.

Miller, M. (2003). Niacin as a component of combined therapy for dyslipidemia. *Myo Clinical Procedures, 78*(6), 735–742.

Mitscher, L. A., Jung, M., & Shankel, D. l. (1997). Chemoprotection: A review of the potential therapeutic antioxidant properties of green tea (*Camellia sinensis*) and certain of its constituents. *Medicinal Research Reviews, 17*, 327.

Mitsuhiro, O., Kazuya, I., Gina, C., Shu, L. C., Malektaj, Y., Tetsuo, N., & Kevin, A. T. (2002). A caffeine diet can alter the mechanical properties of the bones of young ovariectomized rats. *Annuals of Nutrition & Metabolism, 46*, 108–113.

Mooncrieff, R. W. (1951). *The chemical senses* (2nd ed.). London: Leonard Hill.

Motaff, A. C. (1992). *Clarkes isolation and identification of drugs*. London, UK: The Pharmaceutical Press.

Nardone, R., Buffone, E., Move, M., Lochner, P. G., & Tezzon, F. (2004). Changes in motor cortical excitability in humans following orally administered theophylline. *Neuroscience Letters, 355*(1–2).

NNMB (National Nutrition Monitoring Bureau). (1995). Report of second repeat Survey, Rural NNMB Technical report no. 18. Hyderabad, India: National Institute of Nutrition (ICMR).

Ramakrishnan, U. (2002). Prevalence of micronutrient malnutrition worldwide. *Nutrition Reviews, 60*(5), 46–52.

Sehenker, S. (2001). Coffee drinking: Grounds for concern. *British Nutrition Foundation, Nutrition Bulletin, 26*, 5–6.

Siegenberg, D., Baynes, R. D., & Bothwell, T. H. (1991). Ascorbic acid prevents the dose dependent inhibitory effects of polyphenols and phytases on nonheme-iron absorption. *American Journal of Clinical Nutrition, 53*, 537–541.

Tuntawiroon, M., Sritongkul, N., & Brune, M. (1991). Dose-dependent inhibitory effect of phenolic compounds in foods on non-heme iron absorption in man. *American Journal of Clinical Nutrition, 53*, 131–167.

Wang, Z. Y., Agarwal, R., Bickers, D. R., & Mukhtar, H. (1991). Protection against ultraviolet β radiation induced photocarcinogenesis in hairless mice by green tea polyphenols. *Carcinogenesis (London), 12*, 1527–1530.

Weidner, G., & Istvan, J. (1985). Dietary source of caffeine. *The New England Journel of Medicine*, 1421–3131.

Wei, H., Xueshu, Z., Zhao, J.-F., Wang, Z.-Y., Bickers, D., & Lebwohl, M. (1999). Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damages by aqueous extracts from green and black teas. *Free Radical Biology and Medicine, 26*(11/12), 1427–1435.

Woodward, M., & Tunstall-Pedoe, H. (1999). Coffee and tea consumption in the Scottish heart health study follow up: Conflicting relations with coronary risk factors, coronary disease, and all cause mortality. *Journal of Epidemiology and Community Health, 53*, 481–487.

Wu Christine, D., & Wei, G.-X. (2002). Tea as a functional food for oral health. *Nutrition, 18*, 443–444.

Yoshizawa, S., Horiuchi, T., Fujiki, H., Yoshida, T., Okuda, T., & Sugimera, T. (1987). Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of tannin in green tea. *Physiotherapy Research, 1*, 44–47.

You, S. Q. (1993). Study on feasibility of Chinese green tea polyphenols (CTP) for Preventing dental caries. *Chung-Hua Kou Chiang Hsuch Tsa Chih Chinese, 28*, 197.

Zhang, J., & Kashket, S. (1998). Inhibition of salivary amylase by black and green teas and their effect on the intraoral hydrolysis of starch. *Caries Research, 32*, 233.