Journal of Food Biochemistry

Journal of Food Biochemistry ISSN 1745-4514

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BLACK AND GREEN KOMBUCHA TEAS

HOUDA BATTIKH[1,2], KAMEL CHAIEB[2], AMINA BAKHROUF[2] and EMNA AMMAR[1,3]

[1]UR Étude et Gestion des Environnements Urbains et Côtiers, Université de Sfax, LARSEN, École Nationale d'Ingénieurs de Sfax, BP 1173-3038 Sfax, Tunisia

[2]Laboratoire d'Analyse, Traitement et Valorisation des Polluants de l'Environnement et des Produits, Faculté de Pharmacie, rue Avicenne, 5000 Monastir, Tunisia

[3]Corresponding author. TEL: +216-98-41-23-64; FAX: +216-74-27-55-95; EMAIL: ammarenis@yahoo.fr

Accepted for Publication June 20, 2011

doi:10.1111/j.1745-4514.2011.00629.x

ABSTRACT

Kombucha is widely consumed as black tea fermented for 7–14 days. The aim of the present study was to compare the antimicrobial activities of two kombucha beverages originating from green and black teas fermented for 21 days and to characterize the antimicrobial compounds (heat resistance and pH stability). Green and black tea infusions were fermented with a traditional kombucha culture. The resulting kombucha antibacterial/antifungal activities against some pathogenic microorganisms, including human pathogenic bacteria and clinical *Candida* species, were investigated using the agar diffusion method. The results showed interesting antimicrobial potentials of both experimented kombucha teas against the tested microorganisms, except *Candida krusei*. The green fermented tea exhibited the highest antimicrobial potential. Indeed, it showed large inhibition zones against *Staphylococcus epidermidis* (22 mm), *Listeria monocytogenes* (22 mm) and *Micrococcus luteus* (21.5 mm). Furthermore, interesting anti-*Candida* potential was revealed by the reaction of green tea kombucha against *Candida parapsilosis*.

PRACTICAL APPLICATIONS

The black fermented tea is the original and most popular preparation. This research has focused on the investigation of the antibacterial and antifungal activities of the kombucha prepared from green and black teas against a large number of human pathogens to determine and to compare the potential of the two kombucha drinks. The results showed a broad antimicrobial spectrum of kombucha against a range of pathogenic *Candida* involved in several candidoses. Moreover, the data showed that the antibacterial potential of kombucha prepared from green tea was higher than that of the original kombucha tea. Considering the antimicrobial activity demonstrated against a wide range of pathogenic bacteria and clinical *Candida* species, kombucha may be very healthful. As resistance to antimicrobial agents has become increasingly an important global health problem, these findings would be very promising and could be useful as an alternative to current synthetic antimicrobial drugs.

INTRODUCTION

Kombucha is a traditional refreshing beverage resulting from the fermentation of sugared tea with a symbiosis culture of acetic bacteria (*Acetobacter xylinum*, *Acetobacter xylinoides* or *Bacterium gluconicum*) and yeasts (*Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Zygosaccharomyces rouxii, Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, *Brettanomyces lambicus*, *Brettanomyces custersii*, *Candida* sp. or *Pichia membranaefaciens*) (Jankovic and Stojanovic 1994; Mayser *et al.* 1995; Blanc 1996; Liu *et al.* 1996; Balentine 1997; Chen and Liu 2000; Teoh *et al.* 2004). Kombucha fermentation is generally initiated by osmotolerant species, succeeded and ultimately

dominated by acetic tolerant species (Teoh *et al.* 2004). It is characterized by *A. xylinum* activity, producing a floating cellulose pellicle, where cells have been embedded to benefit from close contact with the atmospheric oxygen (Malbaša *et al.* 2008). The tea fungus broth is composed of two phases: a solid floating cellulosic pellicle layer and a sour liquid phase. Acetic acid, ethanol and gluconic acid are the major components of the liquid phase. Other minor constituents such as lactic acid, glucuronic acid, phenolic acids, vitamin B group and enzymes are also present.

By virtue of the numerous reported health-promoting virtues and straightforward safe preparation, this beverage has gained widespread popularity. Indeed, kombucha has been intensively consumed worldwide for a very long time, thanks to its prophylactic and therapeutic properties (Frank 1995; Greenwalt *et al.* 1998, 2000; Dufresne and Farnworth 2000; Yang *et al.* 2009). Particularly, many scientific reports proved that the beverage exerts antimicrobial activity against a broad range of bacteria (Greenwalt *et al.* 1998, 2000; Sreeramulu *et al.* 2001). Furthermore, bacteria and fungi that are present in kombucha form a powerful symbiosis and are able to inhibit the growth of potential contaminating bacteria (Liu *et al.* 1996; Balentine 1997). Indeed, the black tea drink fermented for 14 days was proved to be able to inhibit the growth of *Shigella sonnei*, *Escherichia coli*, *Salmonella enteritidis* and *Salmonella typhimurium* (Sreeramulu *et al.* 2001). Steinkraus *et al.* (1996) reported the antibiotic activity of kombucha against *Helicobacter pylori*, *E. coli*, *Staphylococcus aureus* and *Agrobacterium tumefaciens* mainly related to the acetic acid produced during the fermentation process. Greenwalt *et al.* (1998) reported the antimicrobial potentials of kombucha from both black and green teas on the ninth day of fermentation against *S. aureus, E. coli* serotype H10 (non-pathogenic), *E. coli* serotype H48 (pathogenic), *S. typhimurium*, *Bacillus cereus* and *A. tumefaciens*. They also suggested a similar reaction of these two types of fermented teas without finding any activity against *Candida albicans*. The analyses of the fermented liquid revealed the presence of acetic, lactic and gluconic acids as the major chemical compounds (Frank 1995; Hobbs 1995). Tietze (1995) described the presence of usnic acid, which is an antibacterial agent, in kombucha cultures. More recently, acetic acid has been suggested to be the major antimicrobial agent (Greenwalt *et al.* 1998), and other compounds such as bacteriocins and tea-derived phenolic compounds may also be involved (Sreeramulu *et al.* 2001). Systematic investigation of the antimicrobial activity of kombucha revealed the presence of antimicrobial compounds other than organic acids or proteins (enzymes) produced during fermentation as well as tannins originally present in the tea broth (Sreeramulu *et al.* 2001).

Although green tea can be used for the preparation of kombucha, most of the studies on the antimicrobial activity of drinks were carried out on kombucha prepared from black tea, known as the best substrate (Reiss 1994; Jayabalan *et al.* 2007). Considering the botanical aspect, tea plants belong to the Theaceae family comprising two main varieties: *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (Hara *et al.* 1995a). The first apical leaves are picked from the evergreen shrub and can be processed by different methods. Green tea is readily dried with or without a fixation step to inactivate enzymes (Hara *et al.* 1995b), while black tea, the most popular form around the world, is the result of the oxidation of leaf polyphenols through a multistage enzymatic process fermentation (Hara *et al.* 1995c). Hence, the composition differences between the two types of teas lead to different fermented beverages. Indeed, tea is a very rich complex of over 2,000 different substances (Wheeler and Wheeler 2004). Moreover, the fermentation process contribution leads to several modifications of the final drink composition with biotransformation, secretion and/or degradation of many components. Thus, the resulting kombucha metabolic composition is related to the original substrate in addition to the fermentation duration, ranging from 7 to 10 days at room temperature (Chen and Liu 2000). These parameters need to be considered with more quantitative investigations in order to determine the active compounds and predict the consistent quality of the drink.

The aim of the present study was to evaluate the antifungal and antibacterial activities of kombucha prepared from green and black teas (fermented for 21 days) in order to compare the relative quality of the two fermented beverages. In addition, the characterizations of the heat resistance and pH stability of the antimicrobial compounds were also investigated.

MATERIALS AND METHODS

Starter Culture

The tea fungus used in this study was a Japanese traditional culture kindly provided by Mr. Ian Ogino, an expert of Japan International Cooperation Agency. The starter used was a symbiotic culture between yeast and acetic bacteria, mainly *A. xylinum*.

Preparation of Fermented Tea

Kombucha drinks were prepared using black and green teas (Les jardins du thé, Office Tunisien du Commerce, Tunis, Tunisia). The infusions were prepared after mixing the tea leaves at 10% (w/v) with 20% of sucrose (w/v) in boiling water and steeped for 15 min. The infusions were then filtered using filter paper. The resulting clear filtrate was poured into 250-mL Erlenmeyer flasks. After being cooled to room temperature, the flasks were inoculated with actively growing kombucha culture composed of two portions: a floating cellulose pellicle layer and the liquid broth containing the main

microorganisms involved in the fermentation process. The flasks were covered and incubated at room temperature. After 21 days, the fermented broths were centrifuged at 4,000 rpm, and the supernatants were used for analysis. Unfermented (uninoculated) broths were prepared simultaneously as described earlier, and centrifuged supernatants were used as controls.

pH Determination

The pH of the fermented teas and extracts were measured with an electronic pH meter (Inolab Level 1, WTW Weilheim, Germany).

Target Strains and Cultivation Conditions

The strains species were chosen mainly to represent the most common pathogenic and undesirable microorganisms.

Bacterial Strains and Cultivation. The reference strains of human pathogens used to test antimicrobial activity included gram-positive cocci, *Staphylococcus epidermidis* (CIP 106510), *S. aureus* (ATCC 25923) and *Micrococcus luteus* (NCIMB 8166), and gram-negative bacteria *E. coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *S. typhimurium* (LT2) and *Listeria monocytogenes* (ATCC 19115). The bacterial strains were grown and maintained on Müller-Hinton (MH) agar plates (bioMérieux, Marcy l'Etoile, France) at 37C for 18 h.

Fungous Strains and Cultivation. The human pathogenic yeasts used in the study were isolated from patients suffering from candidosis. These strains were isolated on Sabouraud chloramphenicol agar plates (bioMérieux) and identified by Api ID 32 *C*-test strips (bioMérieux), according to the manufacturer's recommendations. The isolated and identified strains were *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida dubliniensis* and *Candida sake*. The *Candida* strains were grown and maintained on Sabouraud chloramphenicol agar plates at 37C for 24–48 h.

Antimicrobial Activity

The antimicrobial activity was tested by agar diffusion assay (Sreeramulu *et al.* 2001). The Sabouraud chloramphenicol agar medium (45 g/L) was used to evaluate the anti-*Candida* activity, whereas the MH agar medium (38 g/L) was used for the evaluation of the antibacterial activity. The corresponding agar medium (20 mL) was poured into Petri dishes (90 mm in diameter). Suspensions of target strain previously incubated for 24 h were spread on the plates uniformly, and wells of 6 mm in diameter were made with a sterile metal tube by means of a vacuum pump. Kombucha samples were centrifuged at 7,000 rpm for 15 min to remove cell debris (JOUAN Centrifuge BR 4i, Centrifuge BR4i, ThermoFisher, CA). Sterile supernatant was obtained by filtering the supernatant through a sterile microfilter (Microfilter sterile, Sartorius; 0.20-μm pore size, Minisart, Palaiseau, France). Sterile samples (100 μl) were then transferred into the wells in the agar plates previously inoculated with the target strain. The plates were first kept at 4C for 2 h to allow tea sample prediffusion and then incubated at 37C (Sreeramulu *et al.* 2001). The diameter of the inhibition zone was measured after 18 h of incubation.

The antimicrobial activity was evaluated by measuring the growth inhibition zone surrounding the wells. Each experiment was carried out in triplicate and the average diameter ± standard deviation of the inhibition zone was recorded.

For the purposes of control and comparison, acetic acid samples at the same concentration as that of fermented tea after 21 days were prepared and sterilized by filtration and then used for antimicrobial testing, as described earlier for fermented tea samples. In the same way, pH 7 samples of fermented samples were obtained by adjusting the pH with HCl (1 M) or NaOH (1 M). Heat-denatured fermented samples were treated at 120C for 20 min. They were then sterilized by filtration and tested for their antimicrobial activity in the same way as described previously.

RESULTS AND DISCUSSION

Screening of Kombucha Antibacterial Activity

The antimicrobial activity of kombucha tested under different conditions against the studied pathogenic microorganisms is presented in Table 1. Kombucha samples were tested both at their acidic pH as they are produced and after their neutralization (pH 7.0). Heat-treated samples were tested to control the thermostability of the active components in order to confirm their proteic nature. Both kombucha preparations exhibited activities against all the gram-negative and gram-positive tested bacteria. In both fermented teas, the activities were comparable with a slightly higher potential to that noted with the kombucha prepared from green tea. Inhibition diameters ranged from 12 to 22 mm for the green fermented tea, while it varied from 10.5 to 19 mm for black fermented tea.

Kombucha exhibited its strongest antimicrobial effect against *S. epidermidis*, *M. luteus*, *L. monocytogenes* and *P. aeruginosa* (inhibition zone ≥ 18 mm). Acidified green tea was found to be inhibitory toward all of the tested bacteria, but acidified black tea was selectively active only against

TABLE 1. ANTIBACTERIAL ACTIVITY OF KOMBUCHA[a]

Camellia sinensis type	Tested extracts	pH	Inhibition zone diameter (mm)[a] of target bacteria						
			Staphylococcus epidermidis CIP 106510	*Staphylococcus aureus* ATCC 25923	*Micrococcus luteus* NCIMB 8166	*Salmonella typhimurium* LT2	*Escherichia coli* ATCC 35218	*Listeria monocytogenes* ATCC 19115	*Pseudomonas aeruginosa* ATCC 27853
Black tea	Fermented infusion (K_{BT})[b]	2.59	18.5 ± 2.1	14.5 ± 2.1	16.5 ± 0.7	14.0 ± 1.4	10.5 ± 0.4	18.5 ± 2.1	19.0 ± 1.4
	Neutralized kombucha[c]	7.00	N.A.	9.5 ± 0.7	10.0 ± 0.0	N.A.	N.A.	N.A.	N.A.
	Unfermented infusion[d]	5.14	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Acidified infusion[e]	2.59	N.A.	N.A.	N.A.	N.A.	N.A.	27.0 ± 1.4	15.5 ± 0.70
	Heat-denatured Kombucha[f]	2.59	16.5 ± 0.7	13.5 ± 2.1	13.5 ± 2.1	12.0 ± 0.0	13.0 ± 0.0	N.A.	11 ± 0.0
Green tea	Fermented tea (kombucha)[b]	2.54	22.0 ± 1.4	12.0 ± 0.0	22.0 ± 2.8	14.0 ± 1.4	14.5 ± 0.7	21.5 ± 2.1	18.0 ± 0.4
	Neutralized kombucha[c]	7.00	12.5 ± 0.7	N.A.	14.5 ± 0.7	N.A.	N.A.	N.A.	N.A.
	Unfermented tea[d]	5.08	10.0 ± 0.0	N.A.	16.0 ± 0.8	N.A.	N.A.	10.5 ± 0.7	N.A.
	Acidified tea[e]	2.54	27.0 ± 0.0	26.5 ± 0.7	20.5 ± 0.7	18.5 ± 0.7	13.0 ± 0.0	23.5 ± 2.1	13.0 ± 0.0
	Heat-denatured kombucha[f]	2.54	19.0 ± 0.0	16.0 ± 1.4	19.5 ± 0.7	11.0 ± 1.4	12.0 ± 0.0	21.5 ± 2.1	9.0 ± 0.0

[a] Inhibition zone diameter (mean and standard deviation including wells diameter of 6 mm).
[b] Fermented infusion (kombucha) at natural pH value without any adjustment.
[c] Neutralized kombucha: pH 7 fermented infusion adjusted with 1 M NaOH.
[d] Unfermented infusion prepared in the same way as that for making kombucha, and 1 M HCl or 1 M NaOH was used to adjust their pH.
[e] Acidified infusion with acetic acid according to the acidity of kombucha samples.
[f] Heat-denatured fermented infusions were treated at 120C for 20 min.
N.A., no activity revealed.

L. monocytogenes and *P. aeruginosa*. In these cases, the noted inhibition was not the same as that found for fermented tea. Moreover, in most cases, kombucha preparations have not preserved their activity when their pH was adjusted to 7, with the exception of *S. epidermidis* and *M. luteus*. Unfermented tea samples had almost no antimicrobial activity against target microorganisms, except for *S. epidermidis, M. luteus and L. monocytogenes*.

It was also noticed that kombucha teas exerted an antimicrobial activity after thermal denaturation against all the target strains, except for *L. monocytogenes*.

In the case of black fermented tea, the recorded antibacterial activity against *L. monocytogenes* is considered to be related to the organic acid effect. No activities were revealed when the pH was neutralized. Furthermore, the activity was strengthened when the infusion was acidified with acetic acid. However, the behavior of the same strain was different in terms of the green fermented tea, where the activity was improved by tea acidification and such activity remained constant after heat treatment. As a result, the chemical composition of both fermented teas would be completely different.

This finding means that the antibacterial activity observed is not exclusively due to the acetic or other organic acids, but it also implies that other components that are biologically active, such as bacteriocins, proteins, enzymes and tea-derived phenolic compounds, may be involved. This result was in agreement with other studies (Sreeramulu *et al*. 2000, 2001).

Greenwalt *et al*. (1998) tested kombucha that originated from different concentrations of black and green teas and found an antimicrobial activity against gram-positive and gram-negative organisms *(A. tumefaciens*, *Bacillus cereus*, *Salmonella choleraesuis* serotype Typhimurium, *S. aureus* and *E. coli*) and attributed the resulting activities to its acetic acid content. The differences in concentrations between the basic infusions, the fermentation duration, the antimicrobial method and the control extracts used may explain these results. In his study, Greenwalt *et al*. (1998) used the absorbent disc method and the 9-day fermented kombucha and compared only the neutralized and the unfermented form with the fermented extracts.

Screening for Kombucha Antifungal Activity

The results of the antifungal activity of kombucha under different conditions are presented in Table 2. All the pathogenic yeasts tested except *C. krusei* were found to be sensitive to kombucha beverages. However, the spectrum of the antifungal activity was different. In fact, green fermented tea (K_{GT}) was active against *C. glabrata*, *C. parapsilosis*, *C. sake*, *C. dubliniensis* and *C. albicans*. In comparison, black fermented tea (K_{BT}) was able to inhibit *C. tropicalis*, in addition to these yeasts, without having any effect on *C. parapsilosis*

TABLE 2. ANTIFUNGAL ACTIVITY OF KOMBUCHA BEVERAGES[a]

Camellia sinensis type	Tested extracts	Inhibition zone diameter (mm) of target microorganisms[a]						
		Candida glabrata	*Candida parapsilosis*	*Candida tropicalis*	*Candida sake*	*Candida dubliniensis*	*Candida krusei*	*Candida albicans*
Black tea	Fermented infusion (K_{BT})[b]	11.0 ± 1.4	N.A	11.5 ± 0.70	N.A.	12.0 ± 1.4	N.A.	11.0 ± 0.0
	Neutralized kombucha[c]	N.A.	N.A.	N.A	N.A.	N.A.	N.A.	N.A.
	Unfermented infusion[d]	10.5 ± 0.7	N.A.	N.A	N.A.	N.A.	N.A.	N.A.
	Acidified infusion[e]	17.0 ± 0.0	16.5 ± 2.1	N.A	14.5 ± 0.7	18.5 ± 0.7	N.A.	N.A.
	Heat-denatured kombucha[f]	11.5 ± 0.7	N.A.	N.A	N.A	14.0 ± 0.0	N.A.	N.A.
Green tea	Fermented tea (kombucha)[b]	10.5 ± 0.7	15.0 ± 1.4	N.A	9 ± 0.0	13.5 ± 0.7	N.A.	11.0 ± 0.0
	Neutralized kombucha[c]	N.A.	N.A.	N.A	6.0 ± 0.0	N.A.	N.A.	N.A.
	Unfermented tea[d]	N.A.	N.A.	N.A	6.0 ± 0.0	N.A.	N.A.	N.A.
	Acidified infusion[e]	16.0 ± 0.7	11.0 ± 1.4	N.A	15.5 ± 0.7	17.5 ± 3.5	N.A.	N.A.
	Heat-denatured kombucha[f]	11.0 ± 0.0	N.A.	N.A	10.5 ± 0.7	12.0 ± 0.0	N.A.	N.A.

[a] Inhibition zone diameter (mean and standard deviation including wells diameter of 6 mm).
[b] Fermented infusion (kombucha) at natural pH value without any adjustment.
[c] Neutralized kombucha: pH 7 fermented infusion adjusted with 1 M NaOH.
[d] Unfermented infusion prepared in the same way as that for making kombucha, and 1 M HCl or 1 M NaOH was used to adjust their pH.
[e] Acidified infusion with acetic acid according to the acidity of kombucha samples.
[f] Heat-denatured fermented infusions were treated at 120C for 20 min.
N.A., no activity revealed.

and *C. sake*. It was noticed that only *C. glabrata* was inhibited by the unfermented tea. As a result, the fermentation process contributes to the improvement of the antifungal activity.

In most cases, the antifungal activity exerted by acidified tea samples and after heating, depending on kombucha, increased, decreased or even disappeared in the case of *C. albicans*. These results have proved the antimicrobial effects of the acetic acid produced during kombucha preparation. It also highlights its strong effect on the particular yeast and implies the presence of an antimicrobial component other than acetic acid as well as proteins. In addition, neutralized kombucha has not preserved any antifungal activity. These observations are in agreement with the findings of other studies (Sreeramulu *et al*. 2000, 2001), but it should be noted that the antifungal activity is not well studied in the literature. Greenwalt *et al*. (1998) tested the antimicrobial potentials of kombucha prepared from both black and green tea on the ninth day of fermentation against *C. albicans* but did not find any activity. More recently, Sreeramulu *et al*. (2000) demonstrated that kombucha prepared from black tea was able to inhibit *C. albicans* from the sixth day of fermentation to the 14th day.

CONCLUSION

The results of this investigation have shown that after 21 days of fermentation, kombucha still exerts a considerable antimicrobial activity. Thus, all the tested microorganisms were found to be susceptible to the fermented black (K_{BT}) and green (K_{GT}) teas. A relatively higher antimicrobial potential was observed with the green fermented tea (K_{GT}), whereas unfermented teas did not exhibit any antimicrobial properties. Interestingly, kombucha teas exerted antifungal activity against a wide range of pathogenic *Candida* involved in several candidoses. Moreover, this study highlighted the difference between the activities of kombucha prepared from black tea and that from green tea, and demonstrated that each beverage was characterized by a specific spectrum of antimicrobial activity.

It was also shown that the antimicrobial activity revealed was not only the result of acetic acid or organic acids. Other biologically active components may be involved in the observed activity such as bacteriocins, proteins and enzymes in addition to the tea-derived phenolic compounds. Although these results were found to be very promising, further systematic investigations are needed in order to characterize the active component.

ACKNOWLEDGMENT

The authors would like to thank Professor Mickeal Anderson from the Ceramic School in Staffordshire University for his help with English.

REFERENCES

BALENTINE, D.A. 1997. Special issue: Tea and health. Crit. Rev. Food Sci. Nutr. *8*, 691–692.

BLANC, P.J. 1996. Characterization of the tea fungus metabolites. Biotechnol. Lett. *18*, 139–142.

CHEN, C. and LIU, B.Y. 2000. Changes in major components of tea fungus metabolites during prolonged fermentation. J. Appl. Microbiol. *89*, 834–839.

DUFRESNE, C. and FARNWORTH, E. 2000. Tea, Kombucha and health: A review. Food Res. Intern. *33*, 409–421.

FRANK, G.W. 1995. *Kombucha: Healthy Beverage and Natural Remedy from the Far East*, Wilhelm Ennsthaler, Steyr, Austria.

GREENWALT, C.J., LEDFORD, R.A. and STEINKRAUS, K.H. 1998. Determination and characterization of the antimicrobial activity of the fermented tea kombucha. Lebensm. Wiss. Technol. *31*, 291–296.

GREENWALT, C.J., STEINKRAUS, K.H. and LEDFORD, R.A. 2000. Kombucha, the fermented tea: Microbiology, composition and claimed health effects. J. Food Prot. *63*, 976–981.

HARA, Y., LUO, S.J., WICKREMASHINGHE, R.L. and YAMANISHI, T. 1995a. Botany (of tea). Food Rev. Int. *11*, 371–374.

HARA, Y., LUO, S.J., WICKREMASHINGHE, R.L. and YAMANISHI, T. 1995b. Processing tea. Food Rev. Int. *11*, 409–434.

HARA, Y., LUO, S.J., WICKREMASHINGHE, R.L. and YAMANISHI, T. 1995c. Biochemistry of processing black tea. Food Rev. Int. *11*, 457–471.

HOBBS, C. 1995. *Kombucha Manchurian Tea Mushroom: The Essential Guide*, Botanica Press, Santa Cruz, CA.

JANKOVIC, I. and STOJANOVIC, M. 1994. Microbial and chemical composition, growth, therapeutical and antimicrobial characteristics of tea fungus. Mikrobiologija *33*, 25–34.

JAYABALAN, R., MARIMUTHU, S. and SWAMINATHAN, K. 2007. Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chem. *102*, 392–398.

LIU, C.H., HSU, W.H., LEE, F.L. and LIAO, C.C. 1996. The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. Food Microbiol. *13*, 407–415.

MALBAŠA, R., LONČAR, E. and DJURIĆ, M. 2008. Comparison of the products of kombucha fermentation on sucrose and molasses. Food Chem. *106*, 1039–1045.

MAYSER, P., FROMME, S., LEITZMANN, C. and GRÜNDER, K. 1995. The yeast spectrum of the "tea fungus kombucha". Mycoses *38*, 289–295.

REISS, J. 1994. Influence of different sugars on the metabolism of the tea fungus. Z Lebensm. Unters Forsch. A *198*, 258–261.

SREERAMULU, G., ZHU, Y. and KNOL, W. 2000. Kombucha fermentation and its antimicrobial activity. J. Agric. Food Chem. *48*, 2589–2594.

SREERAMULU, G., ZHU, Y. and KNOL, W. 2001. Characterization of antimicrobial activity in kombucha fermentation. Acta Biotechnol. *21*, 49–56.

STEINKRAUS, K.H., SHAPIRO, K.B., HOTCHKISS, J.H. and MORTLOCK, R.P. 1996. Investigations into the antibiotic activity of tea fungus/kombucha beverage. Acta Biotechnol. *16*, 199–205.

TEOH, A.L., HEARDB, G. and COX, J. 2004. Yeast ecology of kombucha fermentation. Int. J. Food Microbiol. *95*, 119–126.

TIETZE, H.W. 1995. *Kombucha: The Miracle Fungus*, 6th Ed., Phree Books, Bermgui, Australia. 72 p.

WHEELER, D.S. and WHEELER, W.J. 2004. The medicinal chemistry of tea. Drug Dev. Res. *61*, 45–65.

YANG, Z.W., JI, B.P., ZHOU, F., LI, B., LUO, Y., YANG, L. and LI, T. 2009. Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. J. Sci. Food Agric. *89*, 150–157.