KOMBUCHA IN EGYPT: IMPORTED AND LOCAL INDUCED CULTURES

ABSTRACT

Kombucha is a sour beverage product obtained by growing consortia of bacteria and yeasts on sucrose sweetened black tea decoction. It is used for its potential health benefits. An imported culture of kombucha and another local induced one were studied. The microbial analysis of both cultures was done. The imported culture appeared homogenic in its microbial structure. It was a consortium of the bacteria *Acetobacter* and *Gluconobacter* spp. and the yeasts *Saccharomyces* and *Pichia* spp. The local culture was induced by inoculation the same (but modified) substrate with water-wash of just cropped organic-cultured ripe tomato fruits. It likely appeared homogenic only after three subcultures on the modified substrate. Both cultures had tow portions. The floating pellicle portion, which principally consisted of cellulosic fibers (31.7% dry weight), and the sour liquid filtrate portion, which finally contained 34-40g acids/l. (expressed as acetic acid), and 0.05-0.1% ethanol (v/v), after 15 days fermentation period.

Key words: Kombucha, Tea fungus, induction, microbial analysis, chemical analysis, *Acetobacter*, *Gluconobacter*, *Saccharomyces*, *Pichia.*

INTRODUCTION

"Kombucha", "Tea Fungus" and "Manchurian tea Mushroom" are synonyms of an association of yeasts and acetic acid bacteria which can ferment a sucrose sweetened black tea decoction **(Beuchat, 1978; Liu *et al*, 1996; Reiss, 1994 and Sievers, *et al.* 1996).**

Besides the biosynthesized microbial cellulose with unique purity and fine structure, the tea fungus beverage contains valuable organic metabolites. Organic acids, minerals and vitamins, antimicrobial substances and other health remedy agents were found **(Fasching, 1995; Frank, 1995; Blance, 1996; Bauer-Petrovska & Petrushevska-Tozi, 2000; Chen & Liu, 2000 and Sreeramulu *et al.* 2001).**

The produced sour and slightly sparkling beverage is consumed world-wide as folk medicinal substances for controversial health claims. Kombucha can lower blood pressure and diabetic blood glucose, increase the immune response, relieve arthritis, psoriasis, chronic fatigue, constipation, indigestion and metabolic diseases and even cure cancer **(Greenwalt *et al*, 2000 and Sreeramulu *et al*, 2001)**

In Egypt, zoogloeal mats of personally imported kombucha are individually circulated among those people seeking for health remedy. So, this mat is called "Life Fish" as an acquired local traditional Egyptian name. In Egypt, kambucha has not been studied from a scientific view. This work is an attempt to verify this microbial phenomenon.

El-Gremi.Sh. M.A. .
Agric. Microbiology Branch, Agric, Botany Dept., Fac. Agric., Kafrelsheikh Univ., Egypt.

MATERIALS AND METHODS

A- Fermentation processing:

Pellicle disc of a readymade imported kombucha culture (kindly obtained from Dr. Abdel Hady, M. A., Prof of Nematology, Fac. Agric., Kafr El-Sheikh Univ.) was suspended in its liquid phase and used to inoculate the prepared sucrose sweetened black tea decoction substrate.

The sucrose sweetened black tea decoction substrate was prepared according to **Chen & Liu (2000).** To boiling distilled water, Lipton black tea (5g/l) and sucrose (100g/l) were added. The mixture was held boiling for 5 min. The solid tea remains were avoided by cheesecloth filtration and the filtrate was immediately dispensed into 3 l capacity clean dry glass jars (1.5 l/jar). After cooling to the ambient temperature, the prepared substrate was inoculated and jars were covered with clean cheesecloth fixed with rubber bands. The fermentation was statically carried out under the ambient temperature 28±2°C for 2 weeks before sub-culturing for new fermentations.

For induction of a local tea fungus culture, separate water–washes of just cropped plant materials (wheat and barely grains as well as cucumber, squash, tomato and grape fruits) were used as initial inoculants of the sweetened tea decoction substrate enriched with equal volume of Frateur's ethanol medium **(Krieg & Holt, 1984)** and acidified to pH 4.5. The inoculated substrate was held in glass jars with the same above-mentioned manner as long as up-surface pellicles arose, and sub culturing was repeated using both the newly arising surface-pellicles and portions of its culture filtrate to inoculate new jars containing the modified sweetened tea decoction substrate. Finally, the obtained induced tea culture, having homogenic pellicle feature likely to that of the important culture, was sub-cultured on the normal sweetened tea decoction substrate

B- Microbial analysis:

Detection of microorganisms during the fermentation process was performed on potato dextrose agar (PDA) medium and glucose yeast extract calcium carbonate agar (GYCA) medium **(Asai *et al*, 1964).** Appropriate sample dilutions were used to inoculate medium surface in Petri-dishes. Inoculated dishes were incubated at 30° C for 3 days. The separated colonies were counted, picked up, purified and microscopically examined. Advanced identification tests of the most dominant bacteria and yeasts to the probable genera were conducted in the guidance **of Holt *et al.*(1994)** for bacteria and **Barnett & Pankhurst (1974)** for yeasts.

C-Chemical analysis:

During the third sub-culturing of both tea fungus cultures (imported and local induced), portions of pellicle-free culture filtrates were periodically withdrawn and filtered through filter paper to be used for chemical analysis. PH value, acetic acid contents, alcohol, sugars and total solids were determined according to **AOAC (1975) and James (1995)** methods.

At the end of the third sub-culturing stable fermentation stages of both imported and local induced cultures, the separated up-surface floating pellicles were air dried before oven dryness at 80°C until constant weight. Ground samples of 3g weight were used to determine contents of the total crude fibers, crude fat, nitrogen and ash using the methods reported in **AOAC (1975).**

RESULTS

Microbial analysis:

Culturing of the imported kombucha on sucrose sweetened black tea decoction resulted in a sour acetic beverage containing up-surface homogenous floating pellicles. Pellicle thickness increased in linear with aging (Fig.1). It reached 34mm at the end of 15 days fermentation period.

Figure (1): The tea fungus culture showing up-surface zoogleal fibrous pellicles floating on vinegary liquid.

In contrast with the homogeneity in microbial combinations of pellicles in case of the imported kombucha culture, the local induced culture (successfully obtained from ripe organic-cultured tomato fruits of cultivar Alisa) had pellicles mixed in its microbial combinations in the early stages of fermentation. It was observed that the thickness of pellicle in these early stages did not exceed 2-3mm with dominance of spore-forming fungi in its mixed microbial structure. Thereafter, homogeneity in the microbial combinations took place by successive sub-culturing in the used enriched and acidified substrate.

Survey of the microbial structure of the imported kombucha culture revealed that the most dominant bacteria showed the morphological and the physiological characteristics of the acetic acid *Acetobacter* and *Gluconobacter* genera, while the dominant isolated yeast genera were identified as *Saccharomyces* and *Pichia* spp. After 2 weeks fermentation period, the ratio of the acetic acid bacteria and the yeasts reached 1:16. On the other hand, the microbial components in the local induced tea fungus culture showed variation along with successive sub-culturing. Firstly, scarce colonies of bacteria and true yeasts were recorded, meanwhile yeast-like and true fungi were dominant. Afterwards, the up-surface growing fungi were enclosed in a thin floating yeast-bacterial biofilm. At this time, ethanol could be smelt and acetic acid bacteria could be detected. After three subsequent sub-culturing in 4.5pH acidified substrate, the microbial consortia likely to that of the imported kombucha culture, solely were present.

Chemical analysis:

Data presented in Table (1) indicate the contents of the up-surface pellicles in both cultures. It is obvious that fibers comprise the major component (31.2-31.7%), while less contents of fats, proteins and ash were recorded (2.4-2.9 %, 4.4-4.9 % and 4.5-4.9%, respectively). No clear differences between pellicle components of the imported culture and the local induced one were observed.

The produced culture filtrates having vinegary smell and taste were periodically tested for characteristics recorded in Figures 2-4. PH values (Fig. 2) gradually decreased over time from 5.8 at the beginning of fermentation to 3.1at the 9th day of fermentation. Then, nearly it stood steady with little increase for the remaining 6 days of incubation period.

Table (1): Primary chemical characterization of the floating pellicle portions obtained from the imported kombucha and the local induced tea fungus cultures.

Contents	% (w/w) of the dried samples	
	The imported culture	The local induced culture
Crude fibers	31.2	31.7
Crude protein	4.4	4.9
Crude fat	2.9	2.4
Ash	4.5	4.9

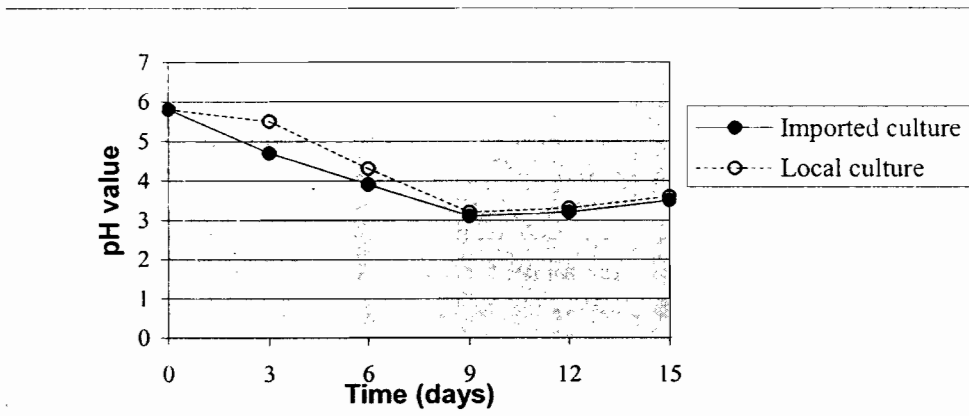

Figure (2): *PH* values of sucrose-sweetened black tea decoction substrate inoculated with the imported kombucha and the local induced tea fungus.

The titratable acidity (expressed as acetic acid) was increased along fermentation time. However, the maximum acid concentrations (35-40g/l) were recorded at the end of 15 days fermentation period (Fig. 3). Ethanol contents increased slowly in the first 3 days followed by rapid increase during the following 6 days. Ethanol contents reached 5% (v/v) on the 9th day of fermentation. A sharp decrease took place during the 6 remaining days when the concentration of ethanol dropped down to ~ 0.4% (v/v) at the end of fermentation period (Fig. 3).

Sucrose, as an invertible non-reducing sugar, showed linear decrease in concentration along with fermentation aging. Its concentrations decreased from 50g/l to 4.8-7.6 g/l (Fig 4). On the other hand, data illustrated in Fig 4 show that reducing sugar contents (expressed as glucose) gradually increased to reach 1.6-2.0 gm/l at the 9th day of fermentation. Afterwards, reducing sugars slowly decreased towards the end of fermentation process reaching 1.1 gm/l.

Regardless to the occurred biomass and volatile portions, the solid substances remained in the fermented substrate reached 43.9 g/l at the end of 15 days fermentation period.

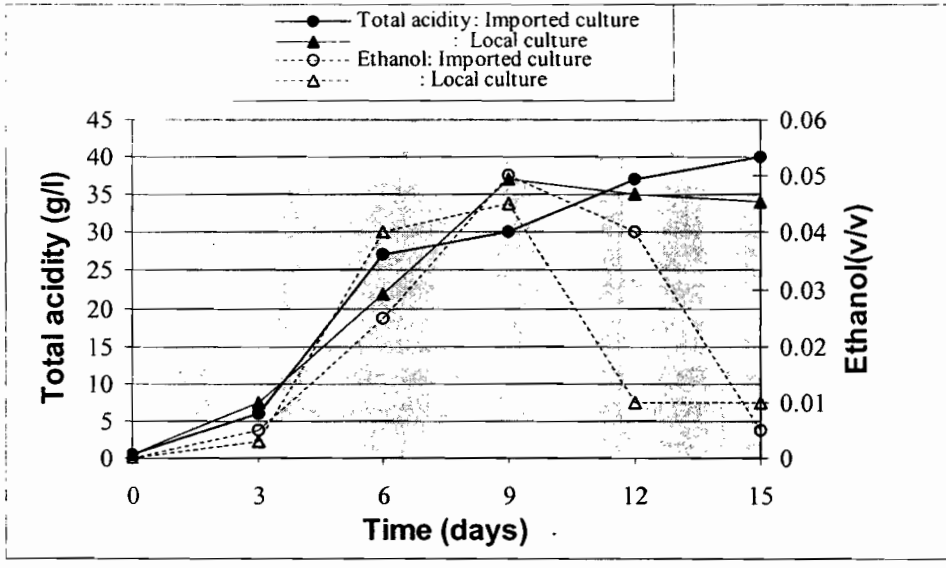

Figure (3): Total acidity (as acetic acid) and ethanol contents in vinegars produced by the imported and the local induced cultures.

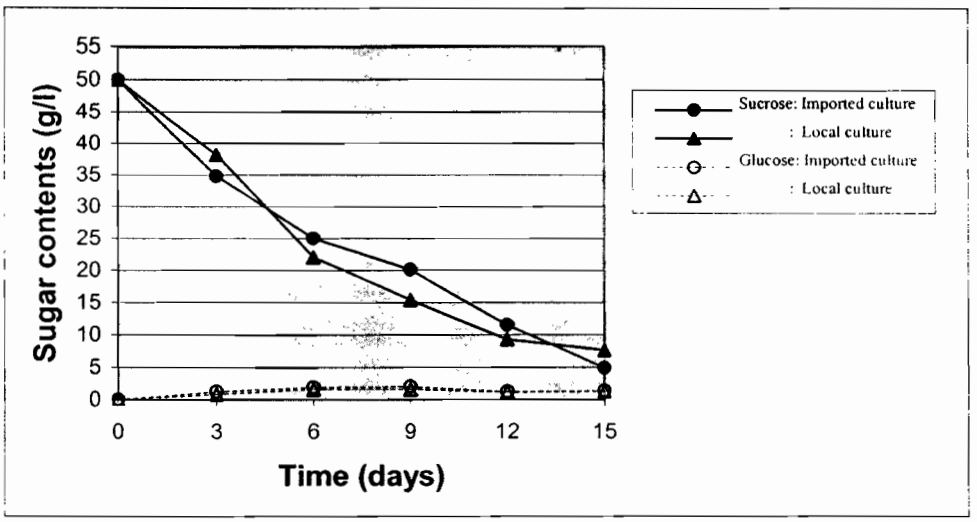

Figure (4): Sugar contents in vinegars produced by the imported and the local induced cultures.

DISCUSSION

Screening the microbial component of bacteria and yeasts consortia in various kombucha cultures performed by numerous researchers resulted in variation of the isolated microbial genera (Liu *et al*, 1996). Although variable compositions of bacterial and yeast combinants were reported in kombucha samples, the major dominant bacterial genera has been reported were *Acetobacter* and *Gluconobacter* in association mostly with *Saccharomyces*, *Pichia* and *Zygosaccaromyces* as the most dominant yeast genera **(Herrera & Caldero´n-Villago´mez, 1989; Liu *et al*, 1996; Sievers *et al*, 1996 and Greenwalt *et al*, 2000).**

In the present study, the imported tea fungus culture appeared homogenic in its microbial structure along over all subcultures. The screened microbial consortium contained solely the acetic acid bacterial genera *Acetobacter* and *Gluconobacter* and the yeast genera *Saccharomyces* and *Pichia*. On the other hand, to obtain the local tea fungus culture, likely homogenic in its microbial structure, three sub-culturing times on the modified sweetened tea decoction substrate were needed. Acidifying the ethanol enriched substrate to pH 4.5 is thought to enable the tea fungus specific microflora to establish and overcome the other competitive contaminants which grew at the initial inoculation. Changes occurred in acidity and ethanol contents of inoculated substrate might help in the microbial group selectivity.

Acetobacter is known to produce cellulosic fibrous pellicles **(Brown *et al*, 1976 and Fontana *et al*, 1991).** In general, cellulose synthesis occurs at a rate that is linear with the cell concentration **(Kadime, 1990)**. Cellulosic pellicle may have multiple functions in the growth and survival of the microorganism in nature. It serves as a floating device to bring the embedded cells in contact with the atmospheric oxygen **(Cook & Colvin, 1980)**. In addition, it provides protection from other mold competitors which used the same substrate. Recently, microbial cellulose has a wide range of human economical applications **(Ross *et al*, 1991).**

This work is the first in Egypt to study the microbial phenomenon, Kombucha. Furthermore, a local tea fungus culture could be induced using plant materials known to be habitable with acetic acid bacteria and yeasts. Other numerous studies are intended to be carried out for verification some of applicable uses of both the floating cellulosic and the liquid filtrate portions of kombucha.

Acknowledgements:-

The author is grateful to Dr. Ghanem, GHA, Dept. of Animal Product, ARC, Egypt, and Dr. Abdel Galeel, MA; Dr. Osman, MF and Dr. El- Sannat, SYA, Dept. Food Technol., Fac. Agric., Kafr El-Sheikh Univ, Egypt for giving opportunities to perform the chemical analysis.

REFERENCES

A.O.A.C.(1975). Official Methods of Analysis of The Association of Official Analytical Chemists.12th edn., ed. Horwitz, W. Benjamin Franklin Station, Washington, DC.

Asai, T.; Iizuka. H. and Komagata, K (1964). The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains. J. General Appl. Microbiol. 10: 95-126.

Barnett, J.A. and Pankhurst, R.J. (1974). A new key to the yeasts. North-Holland Publishing Company- Amsterdam. London.

Bauer-Petrovska, B. and Petrushevska-Tozi, L. (2000). Mineral and water soluble vitamin content in the kombucha drink. Int. J. Food Sci. Tech. 35: 201-205.

Beuchat, L.R. (1978) Food and Beverage Mycology. Avi Publishing Company. Inc. Westport,CT.

Blance, P.J. (1996). Characterization of the tea fungus metabolites. Biotech. Lett. 18: 139-142.

Brown, R. M. Jr.; Willison, J.H.M. and Richardson, C.L. (1976) Cellulose biosynthesis in *Acetobacter xylinum*: Visualization of the site of synthesis and direct measurement of the *in vivo* process. Proceedings of the National Academy of Sciences of the USA. 73: 4565-4569.

Chen, C. and Liu, B.Y. (2000). Clangs in major components of tea fungus metabolites during prolonged fermentation. J. Appl. Microbiol. 89:834-839.

Cook, K. and Colvin, J.R. (1980) Evidence for a beneficial influence of cellulose production on growth of *Acetobacter xylinum* in liquid medium. Curr. Miceobiol. 3: 203-205.

Fasching, R. (1995). Reports on therapeutic success. In: Tea Fungus Kombucha. 9th edn. ed. Fasching, R. Steyr, Austria. Ennsthaler-Verlag.

Fontana, J.D.; Franco, V.C.; de Souza, S.J.; Lyra, I.N. and de Souza, A.M. (1991). Nature of plant stimulators in the production of *Acetobacter xyllinum* "tea fungus" biofilm used in skin therapy. Appl. Biochem. and Biotech. 28/29: 341-351.

Frank, G.W. (1995). Konbucha: Health Beverage and Natural Remedy from the Far East. 9th edn. ed. Frank, G.W. Steyr, Austria. Ennsthaler-Verlag.

Greenwalt, C.J.; Steinkraus, K.H.; and Ledford, R.A. (2000). Kombucha, the fermented tea: microbiology, composition and claimed health effects. J. Food Protect. 63: 976-981.

Herrera, T. and Caldero´n-Villago´mez, A. (1989). Species of yeasts isolated in Mexico from the tea fungus. Rev. Mex. Micol. 5: 205-210.

Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology. 9th ed. Williams & Wilkins, Baltimore, USA.

James, C.S. (1995) Analytical Chemistry of Foods. Blackie Academic& Professional. London, NY.

Kadime, K.; Matsuda, Y.; Ijima, H. and Okajima, K. (1990). Effect of culture conditions of acetic acid bacteria on cellulose biosynthesis. Br. Polym. J. 22: 167-171.

Krieg, N. R. and Holt, J. G. (1984). Bergey's Manual of Systemic Bacteriology. Vol. 1. Williams & Wilkins, Baltimoro/London.

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

Reiss, J. (1994). Influence of different sugars on the metabolism of the tea fungus. Z. Lebensmitt. Untersch. Forsch.198: 258-261.

Ross, P.; Mayer, R. and Benziman, M. (1991). Cellulose Biosynthesis and Function in Bacteria. Microbiological Reviews, 55: 35-58.

Sievers, M.; Lanini, C.; Weber, A.; Schuler-Schmid, V. and Teuber, M. (1996). Microbiology and fermentation balance in a kombucha beverage obtained from a tea fungus fermentation. Syst. Appl. Microbiol. 18:590-594.

Sreeramulu, G.; Zhu, Y. and Knoll, W. (2001). Characterization of antimicrobial activity in kombucha fermentation. Acta Biotechnol. 21: 49-56.

الملخص العربي

ألكمبوشا في مصر :مزرعة مستوردة وأخرى مستحدثة محلياً

شكري محمد علي الجريمي

فرع الميكروبيولوجيا الزراعية، قسم النبات الزراعي، كلية الزراعة، جامعة كفر الشيخ.

ألكمبوشا عبارة عن منتج خمير حمضي المذاق ناتج عن نمو خليط من البكتيريا والخميرة على بيئة شراب الشاي المغلي في الماء والمحلّى بالسكروز .وهذا المنتج يُستخدم لما قد يكون له من فوائد صحية . وتُعتبر هذه أول دراسة لتوضيح هذه الظاهرة الميكروبية في مصر .فقد تم دراسة مزرعة مستوردة وأخرى مستحدثة محلياً للكمبوشا المُنمّاة على البيئة السابقة .وقد أظهرت المزرعة المستوردة تجانساً في تركيبها الميكروبي والذي كان خليطاً من الأجناس البكتيرية *Acetobacter* spp. و *Gluconobacter* spp. وأجناس الخميرة *Saccharomyces* spp. و *Pichia* spp. .

وقد تم استحداث المزرعة المحلية بتلقيح بيئة شراب الشاي المغلي ولكن بعد إدخال بعض التعديلات عليها - بالغسول المائي لثمار طماطم ناضجة ومقطوفة تواً من نباتات مُنتجة بالزراعة العضوية. ولم تظهر هذه المزرعة تركيباً ميكروبياً متجانساً ومشابهاً لتركيب المزرعة المستوردة إلا بعد تجديد الزرع لها لثلاث مرات على بيئة الشاي المعدّلة. وقد كان لكلا المزرعتين جزئين، جزء الغشاء الطافي الذي يتكون بصفة أساسية من 31.7% ألياف، وجزء الراشح وهو سائل حمضي وصل محتواه من الأحماض (معبراً عنها كحمض خليك) إلى 40 -35 جم/لتر ومحتواه من كحول الإيثيل إلى حوالي 0.4 % (حجم/حجم) وذلك بعد فترة تخمر قدرها 15 يوماً.