Journal of Food Technology 5 (2): 164-168, 2007

Functional Potential of a Product from Traditional Biotechnology: Antioxidant and Probiotic Potential of Mbuja, Produced by Fermentation of *Hibiscus sabdariffa* Seeds in Cameroon

[1]B.A. Mohamadou, [2]C.M.F. Mbofung and [3]D. Thouvenot
[1]Department of Food Engineering and Quality Control,
University Institute of Technology, P.O. Box 454, Ngaoundere, Cameroon
[2]Department of Food Science and Nutrition,
National School of Agro-Industrial Sciences, P.O. Box 455, Ngaoundere, Cameroon
[3]Laboratoire de Biodiversité et d'Ecologie Microbienne,
Ecole Supérieure de Microbiologie et Sécurité Alimentaire de Brest, 29280 Plouzané, France

Abstract: This study was designed to determine the functional potential of Mbuja, a traditional condiment produced by fermentation of *Hibiscus sabdariffa* seeds. Antioxidant activity, total phenols and micro-organisms with probiotic action were analysed for samples of 9 origins and compared to that of crude non fermented seeds of *Hibiscus sabdariffa*. The antioxidant activity was assessed by measuring their DPPH-radicals scavenging ability, the total phenols were analysed according to the Folin-Ciocalteu method. Bacteria belonging the lactic acid group and to *Bacillus* genera were screened and characterized. Antioxidant activity varied from 74 to 83% for samples of Mbuja and was always significantly higher than that of crude non-fermented seeds of *Hibiscus sabdariffa*. Total phenols ranged between 0.35 and 0.54 mg 100 g^{-1} for Mbuja of different origins. Bacteria belonging to the *Bacillus* genera were the most important in number isolated while appreciable proportion of lactic acid bacteria was numbered. The increase of both antioxidant activity and phenols could be due the metabolic activity of these micro-organisms. These results suggest that Mbuja is a cheap functional food that provides both antioxidants and probiotics organisms. Its production and consumption could therefore contribute to the consumers health.

Key words: Anti-oxidants, functional, *Bacillus*, lactic acid bacteria, *Hibiscus* seeds

INTRODUCTION

Important changes in food habits essentially due to growing urbanisation in developing countries led to the spread of chronic diseases. People are facing chronic diseases ranging from obesity, diabetes, vascular diseases, arthritic and neurodegenerative diseases, age dependant diseases. This situation is worsened in the last decade by the outbreak of HIV/AIDS pandemic. It is obvious that the increasing poverty among Africans in general and Cameroonian in particular has a synergistic action on the above-cited diseases. One way to reverse this trend is the consumption of affordable foods with health benefits.

With respect to foods of health importance, a growing scientific interest is currently shown to prevent chronic disease by innovative strategies involving nutrition and human health. Epidemiological evidence consistently suggests that increased consumption of some foods offers significant protection against many of these diseases. They provide bioactive components that ensure vital functions and prevent or treat chronic diseases due to oxidative stress. Anti-oxidants and probiotic bacteria are those that focused more attention in most studies.

Antioxidants, which quench free radicals are reported to help cells fighting against oxidative stress and therefore have been linked to disease prevention(Gordon, 1996). They are mainly made of proteins, enzymes and a number of small molecules. The antioxidant activity of the above molecule may be due to their specific scavenging of reactive free radicals, scavenging of oxygen containing compounds or a metal-chelating action. Thus research carried out showed that the protection of living organisms against free radicals toxicity by endogenous and exogenous compounds is also a function of dietary antioxidants known to decrease the risk such chronic diseases as cancer, cardiovascular disorders (Block and Langseth, 1994; Halliwell and Gutteridge, 1999; Wiseman and Halliwell, 1996).

Corresponding Author: B.A. Mohamadou, Department of Food Engineering and Quality Control,
University Institute of Technology, P.O. Box 454, Ngaoundere, Cameroon

On the other hand, probotic are micro-organisms which consumption in some circumstances have been shown to have a beneficial impact on man. A number of groups of microbes have been identified to possess such activity including lactic acid bacteria (Sanders *et al*., 2003). The probiotic effect of micro-organisms include their ability to ease the metabolism of foods, to enhance the immune system and to restore the gastrointestinal flora following an antibiotherapy.

In Cameroon, there is a number of traditional fermented foods that are believed to promote health. It is the case for Mbuja, a condiment produced by fermentation of *Hibiscus sabdariffa* seeds. Preliminary studies revealed that consumers of Mbuja strongly believe in the anti-aging properties of the condiment as well as the low prevalence of cases of stomachaches and chronic diseases among them. Antioxidant and probiotics might be correlated to these assumptions. The present study was therefore carried out to assess the functional potential of Mbuja, especially its antioxidant and probiotics content. Hence, organisms and components responsible of these properties were investigated.

MATERIALS AND METHODS

Sampling: Samples of Mbuja were purchased on local markets of four villages were the condiment is mostly produced and consumed. Three samples were bought in Dzbam; 3 samples were collected in Magoumaz; 2 in Midirey and 1 sample were taken in Gouzda. The condiments were produced by a two steps production process for 10 days. The seeds of *Hibiscus sabdariffa* were cooked (100°C for 3 h), colled at ambiant temperature and fermented in an earthern ware pot for 7 days. Afterwards, the fermenting seeds were pounded and reintroduced in the pot for a further 3 days fermentation. The product were finally dried before preservation.

Total antioxidant activity assessment: Antioxidant activity was determined by the 2.2-Diphenyl-2-Picryl-Hydrazyl (DPPH) method described in (Zhang and Yasumori, 2004) with some modifications. Mbuja content of the methanol extracts of Mbuja were adjusted to 6 mg mL^{-1} (on dry basis), which was chosen as an appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. An aliquot of 1.5 mL of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 mL of Mbuja extract, at 6 mg mL^{-1}. Instead of methanolic extract of Mbuja, pure methanol was used as control. The reaction mixture was vortex mixed and let to stand at room temperature in the dark for 60 min before the decrease in absorbance at 517 nm was measured. Pure methanol was used to calibrate the spectrophotometer. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$AA\% = [(Abs\ control - Abs\ sample)/Abs\ control] \times 100:$$

Total phenol analysis: The content in total soluble phenol was determined according to the method of Folin-Ciocalteu (Swain and Hillis, 1959). The reaction mixture consisted of 0.5 mL of the extract, 5 mL of the distilled water, 0.5 mL of the Folin-Ciocalteu reagent. After a period of 3 min, 1 mL of saturated sodium carbonate solution was added. The 10 mL volumetric flasks were shaken and allowed to stand for 1 h. the absorbance was measured at 725 nm in a UV-Visible spectrophometer. The total phenol content was expressed as mg Gallic acid/g dry extract, mg Gallic acid/100 g dry spices.

Microbiological analyses: Two types of bacteria with important metabolic activity were screened and characterized: *Bacillus* species and Lactic acid bacteria. Spores of *Bacillus* species were isolated on Glucose Agar with Bromocresol Purple (BCP) following heat treatment (80°C for 10 min). Colonies were characterized and identified according to the method described by Gordon *et al.* (1972) based on a set of biochemical tests including enzymatic activity of the isolated strains using appropriate media.

Lactic acid bacteria were isolated on MRS medium and characterized and identified using API 50 CHL kits (BioMérieux, France). Metabolic profiles were read using APILAB software (BioMérieux, France). Lactic cocci were biochemically characterized and identified using specific media according to methods described in the Bergey's manual for bacteriology (Bergey, 1986).

Statistical analyses: Data on the microbial counts as well as antioxidant activity and phenol content of the samples of Mbuja were compared by Analysis of Variance (ANOVA) and the significant differences among means were tested by the Duncan multiple range test using Stat Graphics Plus 5.1 (Manugistic Inc. software, Rockville USA).

RESULTS AND DISCUSSION

Antioxidant activity: The total antioxidant activity of fermented and non fermented seeds of Hibiscus sabdariffa were analysed. The DPPH free radicals

scavenving activities are shown in Fig. 1. All samples of Mbuja showed a higher DPPH radical scavenging activity than crude non fermented seeds of *Hibiscus sabdariffa*. Value for crude seeds was 56.7% of DPPH inhibition while that of different samples of Mbuja ranged between 74 and 84%. The total antioxidant activity of some condiments. Fermentation increased significantly ($p<0.05$) the total antioxidant activity of the seeds.

Recent studies reported the effect of fermentation on the antioxidant activity of some food products. The yeast fermentation increased by 5.5 times the antioxidant activity of soy flour (Li, 2004). Similarly, Young-Hee *et al.* (2005) observed an increase in DPPH radical scavenging in soybeans fermented by lactic acid bacteria. The antioxidant activity is due to compounds as different as vitamins, mineral and phenols (Marc *et al.*, 2004). In addition to these compounds, evidence of implication of peptides and coenzymes has been brought by recent studies. Hence, food protein hydrolysates have been shown to have antioxidant activities attributed essentially to low molecular peptides produced during hydrolysis of food protein (Rhee *et al.*, 2004). For the case of Mbuja, the microorganisms associated with the fermentation of *Hibiscus sabdariffa* seeds may have influenced their total antioxidant activity. Their metabolism could have influenced the total antioxidant activity of Mbuja, in addition to the effect of other components such as phenols. In this respect, biochemical characterization of strains belonging to the genus *Bacillus* isolated in samples of Mbuja brought evidence of the proteolytic activities of most strains.

Hydrolysis of protein was evaluated *in vitro* on casein and gelatine. The diameter of the clearing zone around the colony of bacteria was measured upon incubation. Measures revealed different but significant enzymatic activity of the 26 strains isolated (Table 1). A few isolates (S5, S7 and S12) did not hydrolyse casein while S6 and S10 were not proteolytic on casein. Given the high protein content of the seeds of *Hibiscus sabdariffa*, this important proteolytic activity could result in the increase of free peptides and amino acids thus contributing to the increase of total antioxidant activity as shown by Rhee *et al.* (2004).

Total phenols content: The total soluble phenols were analysed. Results presented on Fig. 2 reveal significant

Table 1: Proteolytic activity of *Bacillus* isolates on gelatine and casein

	Diameter of the clearing zone (mm) after 24 h	
Strain	Gelatine	Casein
S1	18^{a}	10^{b}
S2	18^{a}	5^{c}
S5	18^{a}	15^{a}
S6	12^{b}	0^{d}
S7	18^{a}	15^{a}
S8	6^{c}	5^{c}
S9	6^{c}	5^{c}
S10	6^{c}	0^{d}
S11	12^{b}	5^{c}
S12	18^{a}	15^{a}
S13	12^{b}	10^{b}
S14	6^{c}	5^{c}
S15	12^{b}	5^{c}
S16	6^{c}	5^{c}
S17	12^{b}	10^{b}
S18	12^{b}	5^{c}
S19	12^{b}	10^{b}
S20	6^{a}	5^{c}
S21	6^{a}	5^{c}
SX	6^{a}	10^{b}
SY	6^{a}	10^{b}
SAc	6^{a}	5^{c}

Values on the same column with the same letter as superscript are not significantly different at the level of 5% according to Duncan test

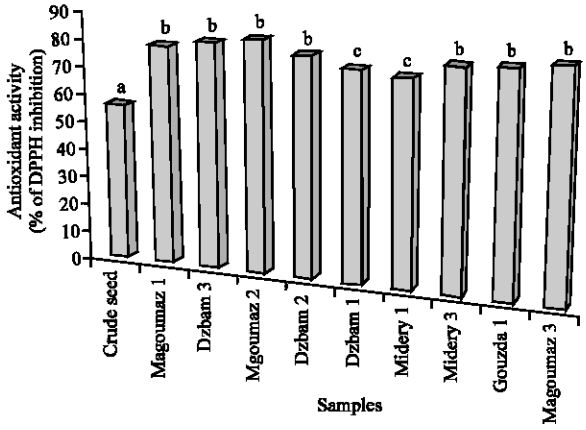

Fig. 1: Total antioxidant activity of samples of Mbuja compared with that of non-fermented seeds. Histograms with different letters as subscript are significantly different by ANOVA

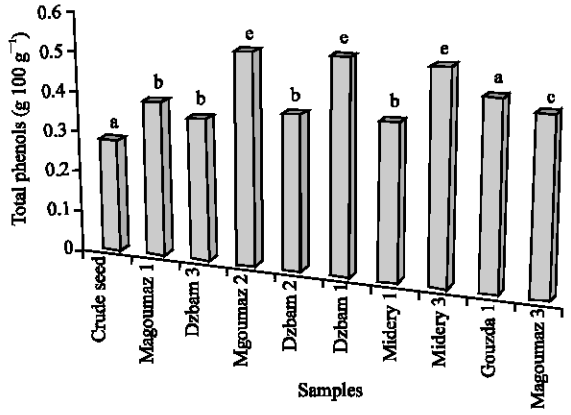

Fig. 2: Total phenols of samples of Mbuja compared to that of crude seeds of Hibiscus sabdariffa. Histograms with different letters as subscript are significantly different by ANOVA

difference between crude seeds of *Hibiscus sabdariffa* and different samples of Mbuja obtained by fermentation of these seeds. A mean of 0.28 g 100 g^{-1} of phenols was obtained for crude seeds whilst that of the fermented seeds were comprised between 0.38 and 0.52 g 100 g^{-1}.

The most important values of phenol were obtained for the Mbuja from Magoumaz 2, Dzbam1 and Midirey3. Fermentation has therefore increased total phenols in the seeds of *Hibiscus*.

Its has been proven that fermentation is very efficient in bio-enrichment of foods in different components such as vitamins (Streinkraus, 1997). Therefore, fermentation was associated w ith biosynthesis of L-ascorbic (Hong *et al*., 1996) and beta-carotene and vitamin E (Takeyama *et al*., 1997). Hence, the increase of total phenols could be due to the synthesis of these antioxidant vitamins also included in the group of polyphenolic substances (Marc *et al*., 2004). The role of phenolic compounds in developing antioxidant activity is well known. However, in the present study, no statistically significant correlation was found between total phenols and antioxidant activity, meaning that other compounds contribute to the DPPH radical scavenging of Mbuja. These other compounds might possibly derive from microbial activity during the fermentation process.

Microbiological analyses: Two groups of bacteria with possible link with antioxidant activity and probiotic potential were screened and characterised: Lactic acid bacteria and *Bacillus* sp.

The analysis of *Bacillus* spores revealed counts comprised between counts of spores of *Bacillus* revealed 2.6×10^{5} and 6.2×10^{7} spores g^{-1} (Fig. 3). There was a significant difference between samples regardless to their origin and producer. Eight species were identified upon biochemical characterization. The *Bacillus* isolated belonged to the species *subtilis* (7%), *pumilus* (19%), *brevis* (15%), *polymyxa* (4%), *licheniformis* (15%), *laterosporus* (27%), *cereus* (7%) and *circulans* (6%).

Besides *Bacillus* sp., lactic acid bacteria were been identified as important part of the microflora of Mbuja (Fig. 4). The total counts of lactic acid bacteria ranged between 6.6×10^{7}UFC g^{-1} for the sample from Dzbam 1 and 5.8×10^{5} UFC g^{-1} for that of Magoumaz 1. However, the proportion of these bacteria did not vary significantly for 10 of the 12 samples studied. Eight gram positive and catalase negative strains were isolated on MRS media. Biochemical characterization using API 50 CHL galleries and tests described by the Bergey's Manual of Determinative Bacteriology (1986) showed that these strains belonged to three species: *Lactobacillus bulgaricus*, *Leuconostoc mesenteroides dextranicum* and *Pediococcus pentosaceus*.

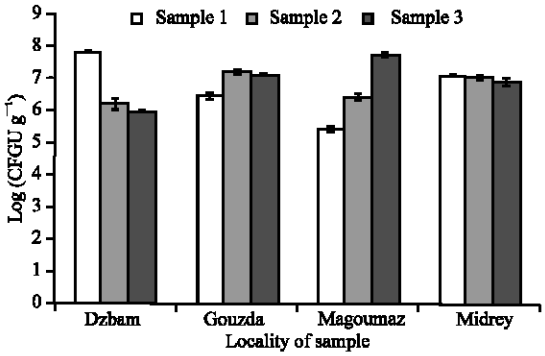

Fig. 3: Counts of *Bacillus* in the samples of Mbuja from different localities

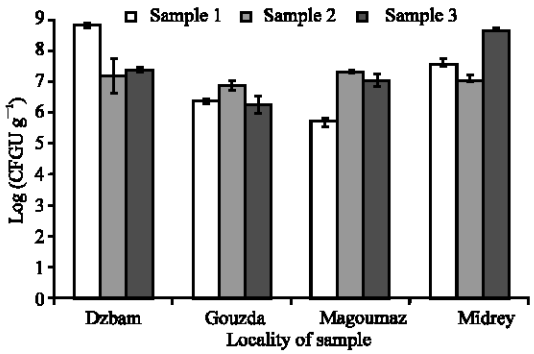

Fig. 4: Counts of lactic acid bacteria in the samples of Mbuja from different localities

Recent studies have shown that consumption of certain live micro-organisms are beneficial to man (Sanders *et al*., 2003). Hence, the contribution for the maintenance of health in humans of these bacteria, known as probiotics, is widely studied. These include *Lactobacillus* species but also sporeformers such as *Bacillus* and *Brevibacillus* (Sanders *et al*., 2003). In the present study, both groups of bacteria were isolated and appeared to be the most important microflora associated with the fermentation of *Hibiscus sabdariffa* seeds. *Bacillus* species were identified as the main flora occurring in a number of other traditional condiments produced by fermentation of proteinaceous seeds (Ndir, Odunfa, Steinkraus).

CONCLUSION

The potential of Mbuja as functional food was evaluated in this study. Analyses revealed the important antioxidant activity and probiotic potential of all samples investigated. The antioxidant activity of Mbuja is probably due to the combine composition in bioactive compounds and the effect of fermentation carried out by *Bacillus* and lactic acid bacteria. The traditional

biotechnology proved to be beneficial to Mbuja consumers since it improves the functional potential while providing bacteria of probiotic potential. This probably explains allegation by villagers and main consumers that Mbuja maintain health and strength. The present study could be confirmed by *in vivo* tests for antioxidant and probiotic activity of the condiment. The use and consumption of this condiment would therefore be advised and encouraged upon optimised and controlled traditional fermentation.

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