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Microbiological Quality of Locally Produced *Spirulina* in Comparison with a Commercial Sample

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ABSTRACT

This study was carried out to determine the microbiological load of the fine powder of the locally produced *Spirulina* in Saudi Arabia in comparsion with a commercial sample. Biological raw materials can be contaminated with microorganisms and, to make them suitable for commercialization, the microbial quality is necessary to be achieved. The microalgae industry has developed to its current status by providing a safe and nutritious product for the human supplement market as well as the animal and aquaculture feed markets. The vast majority of this microalgae biomass is produced from *Spirulina, Chlorella,* and *Aphanizomenon flos-aqua*. *Spirulina* are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira* are consists of about 15 species. Of these, *Arthrospira platensis* is the most common and widely available *Spirulina* and most of the published research and public health decision refers to this specific species. *Spirulina* has been marketed and consumed as a human food and has been approved as a food for human consumption by many governments, health agencies and associations of about 90 countries.

Key words: *Spirulina,* Microbiological load, Microalgae biomass.

INTRODUCTION

Biological raw materials can be contaminated with a great number of bacteria and fungi (Soriani *et al*., 2005), due to the medium (water, soil) in which they grow. This contamination makes them inadequate for food, pharmaceutical and cosmetic applications (Soriani *et al*., 2005). Until two decades ago, taking vitamin or mineral supplements - especially herbs - was considered to be at the fringe of respectability by mainstream medicine. But by 2005 the nutraceutical and dietary supplement industry had become a $182 billion global market. Sales of all dietary supplements, including vitamins, minerals, herbs, and similar compounds grew 2.6 % annually from $8.8 billion in 1994 to $20.3 billion by 2005.

The dietary supplement industry is made up out of a variety of products many of them are heterogeneous products varying greatly both between the product types and within a particular product. For example, analysing *Spirulina* (*Arthospira*) from various producers and distributors shows marked variations in the vitamin, protein, mineral, pigments, and microbial quality. In some cases there are significant variations between different batches produced by the same producer (Johan, 2003). With a growing number of ingredients in these products, it has become increasingly important to ensure they are free of potentially harmful microbial contaminants at the manufacturing level.

The microalga (*Cyanobacterium*) *Spirulina* (*Arthrospira*) is one of the most important microorganisms due to its suitable content of protein, vitamins, minerals, pigments and phytonutrients. *Spirulina platensis* was the first *Cyanobacterium* to be commercially cultivated using modern biotechnology (Hu, 2004). It has been used as a food supplement for human and animal nutrition

(Ciferri, 1983; Jassby, 1988a; Richmond, 1988; Belay, 1997; Al-Batshan *et al*., 2001; Becker, 2004). The final product of *Spirulina* must meets the quality criteria set by different producers and organizations in order to be used as a food supplement in local and international markets (Jassby 1988a; Becker, 1988, 1994, 2004 Belay, 1997). *Spirulina platensis* this amazing blue-green algae, more and more used as medicine and superfood (Belay and Amha, 2002; Li *et al.,* 1997), containing the most remarkable concentration of nutrients known in any food, plant, grain or herb.

In Saudi Arabia, the interest in *Spirulina* started in 1999 when the Arabian agricultural Service Company (ARASCO) established a small farm of this microalga (Al-Homaidan, 2002). Microbiological contamination of Biological raw materials, that could occur either during pre or post harvest processing, is of serious concern (Soriani *et al*., 2005). Therefore, the assurance of microbial quality according to international requirements (UNIDO, 1984) is necessary to commercialize these biological materials. Much has been written about the chemical composition and nutritional properties of microalgae (e.g., Becker, 1995; Payer et al., 1980). These earlier reports deal specifically with microalgal biomass as single cell protein source and this was especially pertinent in the 80's when alternate protein sources were being sought. In the present work, the microbiological load of the fine powder of the locally produced S*pirulina* in Saudi Arabia were compared with a commercial sample.

EXPERMENTAL

Sample preparation

10 grams of the two *Spirulina* samples were emulsified with 90ml of buffered peptone water, by heating with frequent agitation in water bath at 45°C for 15 minutes.

Microbiological analysis

The microbial load of the samples was analyzed by the quantitative assay of viable microorganisms following the technique of pour plate. Samples were used to prepare serial dilutions according to standard methods (AOAC, 1990) with modifications. Aerobic plate counts (APCs) were enumerated after plating with double case agar (PCA) and incubating at 35±2°C for 48 h to determine the total plate counts for bacteria in a procedures mentioned below. Also a numbers of different selective media were used to detect pathogenic bacteria

Total aerobic viable count

1. Take 1 ml from the above preparation and added 9 ml of tryptone soy broth. This is 2nd dilution 1:100.
2. From the above dilution I toke 1 ml of sample and added to 9 ml tryptone soy broth. This is 3rd dilution 1: 1000.
3. Take 1 ml from each dilution above in duplicates plates and added 9 ml pre melted nutrient agar which was cooled down to 45°C .
4. Tilt the plates back and fort to get a homogenous mixture.
5. Leave the plates until it cools.
6. Incubate the plates in inverted position for 48 hours in 35±2°C

Determination of pathogenic organisms

1. From the initial dilution above, inoculate 3 tubes with 1 ml of sample.
2. Label each tube with A, B, C respectively.
3. Incubate all tubes in 35±2°C for 24H. Observe for turbidity.
4. After 24 hours sub-cultured each tube in blood agar plates using isolation streaking.
5. Incubate the plates in 35±2°C for 24 hours.
6. Result: growth in all blood agar plates.
7. Perform gram stain direct from aerobic blood agar plates.
8. Observation: gram positive rods = Bacillus species
9. Subculture the growth from blood agar to different selective media.

RESULTS AND DISCUSSION

Microbial load has been determined for bacteria in both commercial and local sample. Regarding local sample it can be noted that the number of contaminant colonies as presented in table (1) revealing the total aerobic viable counts from the three different dilutions prepared. From this results its clear that the microbial load of the locally produced *spirulinain* in Saudi Arabian is

almost similar to the commercial sample. This is may be due to the alkalinity of the growth medium, the microbial load of *S. platensis* cultures has been reported to be one order of magnitude lower than that of eucaryotic algae, such as *Scenedesmus acutus*, which grow in acid media (Becker and Venkataraman, 1982). Mono-specific mass algal production is subject to contamination from a variety of organisms. Such contamination is inevitable because sterility is lost in large-scale production systems, being less of a problem in closed reactors (Johan, 2003).

However, a quantitative study of the bacterial flora associated with open-pond cultures of *S. platensis* and *S. maxima* has shown that the contaminating bacteria may account for ca. 1% of the total biomass (Materassi, 1980). If the trichomes are washed repeatedly with sterile physiological solution, the bacterial contribution to the total biomass becomes negligible (<10^3 bacterial cells per trichome) (Riccardi, 1976). Thus, harvesting of cultures by filtration or centrifugation followed by washing may result in biomasses that contain insignificant amounts of bacterial contaminants.

Microbiological investigations of crude samples obtained from many different areas have indicated the presence of aerobic and anaerobic bacteria, and fecal streptococci have been isolated from *S. maxima* harvested from Lake Texcoco (Jacquet, 1976). As expected, the number of all viable bacteria, including those that may represent a danger to human health, decreases in the dried samples even in those processed by sun drying (Becker and Venkataraman, 1982; Jacquet, 1976). This is totally agreed with our results since the sun light was used in drying the local sample

The bacterial load in indoor alga cultures is much less if it compared to the open basin algal cultures exposed completely to the environment (Mahadevaswamy and Venkataraman, 1981). The contamination is possibly from water and also from air. The excretion of certain organic substances by the algal cells into the media which may support bacterial growth has been reported in algal cultures that are grown in simple inorganic media (Vela and Guerra, 1966).

Bacteria are always present in non-sterile cultures even in cultures with obligate photoautotrophs, because the latter release many different organic compounds of potential use to heterotrophic bacteria. Since it is impossible to screen for all bacteria, so-called indicator organisms are mostly used, which include enumeration of *Escherichia coli*, *Salmonella* spp., *Shigella spp*.

Table 1: Total aerobic viable count

Dilution	24 H *CFUs/ml*		48H *CFUs/ml*	
	Commercial sample	Local sample	Commercial sample	Local sample
1:10	6	8	6	8
1:100	0	0	0	0
1:1000	0	0	0	0

Table 2: Determination of pathogenic organisms

Selective media	Observations	
	Commercial sample	Local sample
Mac Conkey agar	No growth	No growth
Manitol Salt Agar	No growth	No growth
Salmonella Shigella agar	No growth	No growth
Sabouraud Agar	No growth	No growth

staphylococci and *micrococci spp*. A standard plate count is often performed and the accepted requirement is that it should be either less than 100 000 or 200 000 g^{-1} (Johan, 2003).

Table (2) presents the determination of pathogenic bacteria using different selective media for those kinds of organisms. In indoor cultures, *coliform* group of organisms was not detected also *Salmonella* spp., *Shigella spp*., *staphylococci* and *micrococci spp*. Since there are no growth was detected, a result indicating the good microbiological quality of the sample and the adequate hygiene used during culturing and processing of the product.

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