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Microbiological evaluation of gum Arabic (*Acacia senegal* Gum) from tree to store and during processing

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Abstract
The microbial load and hygienic status of gum Arabic was determined at various stages of production storage and processing. The mean value for total bacterial viable count ranged from 1.3×10^2 to 4.6×10^4 Colony Forming Unit/gram (CFU/g), viable mould count was 0.3×10 to 10.1×10^3 CFU/g and for aerobic spore-forming bacteria the mean value ranged from 1.3×10 to 2.6×10^4 CFU/g. The general trend in that the microbial load of gum Arabic decreases as one proceeds from time of exudation on the tree to the final processing stages. Hygienically, all gum samples collected aseptically from the trees were free of *Staphylococcus aureus*, *Salmonella spp* and Coliforms. *Salmonella spp* and Coliforms were first detected on the gum samples collected by ferments while still in the field. *Staphylococcus aureus* was first detected in samples from the village market. *Escherichia coli* was not detected in any sample of gum arabic. In addition to *Salmonella spp*, *Staphylococcus aureus* and *Bacillus spp*, other bacterial genera identified in gum arabic include *Pseudomonas spp*, *Moraxella spp*, *Enterobacter spp*, *Providencia spp*, *Serratia spp*, *Klebsiella spp*, *Proteus spp* and *Cedecea spp*. The fungal genera identified were *Aspergillus spp*, *Penicillium spp* and *Curvularia spp*. Yeasts were not detected in any gum arabic sample.

Keywords: Microbiological, Gum Arabic, *Acacia senegal*

Introduction
Sudan is a large country, extending over many climatic regions and different environments. It is considered the first country in gum arabic production, as it produces annually more than 80% of the total world production (Taha, 1999) [20].

Gum arabic is defined as the dried exudates, obtained from the stems and branches of *Acacia senegal* (L.) Willd (called in Sudan, hashab) or related species of *Acacia* (family: *leguminoseae*), which is extensively found in the so-called gum belt. Gum arabic contains basically complex polysaccharides of high molecular weight, acids and salts (Leung, 1980) [15]. The gum arabic production channels from the stages of tapping and collection through marketing up to storage and processing provide good chances for contamination with microorganisms. In order to give improved functional properties of gum and to meet specifications required by gum manufacturers, by ensuring a constant product quality, spray-drying technique of gum arabic was applied in Babanousa dry milk factory in 1969. Unfortunately, this trial stopped, partially because of unacceptable microbiological quality and complaints from international customers. For example, one international company showed that total microbial count was 16000/g whereas the acceptable number was only 1000/g (Dirar, 1976) [4].

Khalid (1984) [13] found that the total viable count of bacteria per gram of gum to range from 4.8×10^5 to 9.9×10^6 CFU/g. Gum arabic samples picked from trees, local market and in the store had viable count ranging from 5.9×10^3 to 1.3×10^5 CFU/g, 2.9×10^4 to 7.7×10^5 CFU/g and 1.7×10^4 to 3.1×10^6 CFU/g, respectively, in Elobied area (Osman, 1998; Karamalla, 1999) [19, 12]. Viable count of crude gum arabic samples ranged from 4.5×10 to 8.4×10^4 CFU/g in one study (Ismail, 2000) [10] and did not exceed 8.2×10^3 CFU/g in another (Mahdi and Mohamed, 2006) [16]. The total viable count of fungi/g of gum arabic ranged from 2.0×10^3 to 2.0×10^5 CFU/g (Khalid, 1984) [13].

Viable fungal count of gum arabic samples picked from trees, local market and in the stores ranged from 2.3×10^3 to 7.0×10^3CFU/g, 3.1×10^4 to 4.2×10^5CFU/g and 2.6×10^4 to 3.8×10^5CFU/g, respectively, in Elobied area (Osman, 1998; Karamalla, 1999) [19, 12]. In another study, fungal count of crude gum arabic samples ranged from 3.0×10 to 1.5×10^2CFU/g (Ismail, 2000) [10]. This study aimed at determining the load of various microbial groups in gum arabic at various stages of production, storage and processing with the intention of finding out the role of each stage in determing the hygienic status of this important commodity.

Materials and Methods

Materials: The samples of gum arabic for this study were collected from West Kordofan state (the villages of El Nahud, Eyal Bakheet and Wad Banda) and North Kordofan state (Shekan, Um Rwaba, Nawa, El Rahad and Damukya), as well as from Elobied crops market, Gum arabic Company (GAC) stores at Elobied town and Khartoum Gum Arabic Processing Company (GAPC) at Khartoum town. A random sampling technique was adopted in sample collection at the following points: After exudation, after collection, at village, at crops market, after 3-month storage, after one year storage, kibbled gum, powdered gum and spray-dried gum.

Microbiological methods: Samples for microbial testing were collected by glove-covered hands using sterile polythene bags and stored at 4-10ºC pending use (Kiss, 1984) [14]. The microbiological methods followed were those described by Hobbs (1978) [8], Harrigan and McCance (1976) [7], FAO (1992) [5] and Harrigan, (1998) [6]. All the media used were Oxoid products. The viable bacterial count was done using the pour plate method using plate count agar. Samples for spore-former count were done by pour plate using nutrient agar after samples pasteurized at 80ºC for 10 minutes. Fungal count was done by pour plate using yeast extract agar.

Coliforms and *E. coli* were determined as the most probable number (MPN). *Salmonella spp* was detected by enrichment in nutrient broth, selenite cystine broth, plating on bismuth sulfite agar and checking with triple sugar iron agar. *Staphylococcus aureus* was detected by growth on staphylococcus medium No.110. Further verification tests were carried out on suspected isolation for purposes of confirmation. Identification of bacteria was done by the traditional biochemical test (Cowan and Steel, 1974) [3] and by the API system. Fungi were identified according to Singh *et al.* (1991).

Results and Discussion

Total viable counts (TVC) of bacteria: Total viable count (TVC) of bacteria of 54 samples taken during the production of gum arabic at different phases showed that the mean value was 4.6×10^4CFU/g at village market and the lowest value was 1.3×10^2CFU/g of spry-dried gum samples (Table 1). The bacterial load during the present study was generally not high, but there was increase in the bacterial load of samples taken after collection in field from after exudation. However, there was some increase in the bacterial load during transport from field to the village market. This could be due to accumulation of high moisture content which led to microbial growth. The microbial load decreased at the crops market, on storage and at processing steps. This decrease could be due to the low moisture content and to the death of the bacteria by the sun. A sharp drop in TVC of bacteria after processing of kibbled gum, powdered gum and spray-dried gum was observed. This drop may be due to low moisture content and to the death of the bacteria by the high processing temperature.

Total viable counts (TVC) of moulds: Generally, no signs of definite spoilage by moulds were detected since TVC of moulds of value < 1.0×10^2 CFU/g. This is considered to be negligible in food (Jay, 1986). The mean value of TVC of moulds through 9 sampling points at different phases of gum arabic production showed that the highest value was 10.1×10^3 CFU/g after collection of gum nodules and the lowest value was 0.3×10 CFU/g in spry-dried gum samples (Table 2). In general, contamination with moulds of samples taken during the exudation of gum nodules and after collection and at crops market was relatively high compared to the other phases of gum production. This could be attributed to the presence of millet and sorghum fields in the same area; contamination could originate from these millet and sorghum fields. Also there was a decrease in the TVC of moulds at the village market. This decrease could be due to the drying effect and low moisture content. But there was a little increase of mould load at crops market, which could be due to the handling of gum arabic. There was a decrease in count at the storage phases and at processing steps which could be due to the low moisture content and drying effect that lead to a stop of mould growth and to the death of some sensitive species.

Table 1: Total viable counts of bacteria (CFU/g) of samples drawn during different stages of gum arabic (values for 6 samples in each stage)

Phases	Minimum	Maximum	Mean
After exudation	1.2×10^3	4.0×10^4	2.6×10^4
After collection	2.8×10^3	2.2×10^4	1.2×10^4
At village market	2.0×10^3	9.0×10^4	4.6×10^4
At crops market	5.0×10^2	8.0×10^3	4.3×10^3
After 3months storage	3.1×10^3	8.0×10^3	5.6×10^3
After one-year storage	3.1×10^3	4.5×10^3	3.8×10^3
Kibbled gum	Nil	6.2×10^2	3.1×10^2
Mechanical powder	5.0×10	7.6×10^2	4.1×10^2
Spray-dried gum	Nil	2.5×10^2	1.3×10^2

***** Values for 6 samples

Table 2: Total viable mould counts (CFU/g) of samples drawn during different stages of gum arabic handling*

Phases	Minimum	Maximum	Mean
After exudation	8.0×10^2	1.7×10^4	8.9×10^3
After collection	1.0×10^2	2.0×10^4	10.1×10^3
At village market	2.0×10^2	8.0×10^2	5.0×10^2
At crops market	2.0×10^2	2.0×10^4	10.1×10^3
After 3months storage	1.0×10^2	2.0×10^3	1.1×10^2
After one-year storage	3.0×10^2	6.0×10^2	4.5×10^2
Kibbled gum	Nil	1.8×10^2	9.0×10
Mechanical powder	Nil	6.6×10	3.3×10
Spray-dried gum	Nil	0.6×10	0.3×10

***** Values for 6 samples

Total viable counts (TVC) of spore-forming bacteria: The spore-forming bacteria were detected in all the 54 samples of gum arabic at different phases and the result showed that the highest mean value was in samples of gum after 3-month and after one-year storage (2.6×10^4 CFU/g) and the lowest mean value at powdered gum samples (1.3×10 CFU/g) (Table 3).

It was noted that spore-formers showed high differences between the phases of gum production. The results generally show a steady increase in the spore-formers load from exudation up to the stage of storage. The present results show in some cases that TVC of spore-formers is higher than the TVC of total bacteria. This increase could be due to the fact that some spore-formers do not grow without being activated first by heat treatment, as happens in pasteurization of the sample for spore-former count. Also there was a sharp drop in the count of spore-forming bacteria after processing of kibbled gum, powdered gum and spry-dried gum. This result and the fact that no spore-forming contamination took place during the milling step may be due to implementation of proper hygienic conditions.

Detection, isolation and identification of some health indicators: Detection of some health indicators was carried out to evaluate the hygienic conditions applied during the different phases of gum arabic production. No *Staphylococcus aureus*, *Salmonella spp*, coliforms or *E. coli* was detected in all samples taken after exudation of gum nodules. This finding indicates good hygienic conditions in this phase of gum production. No *Staphylococcus aureus* or *E. coli* were detected in the phase after collection in the field, but *Salmonella* and coliform bacteria were detected. This finding indicates contamination through handling of gum nodules.

Table 3: Total viable counts of spore-forming bacteria (CFU/g) of samples drawn during different stages of gum arabic handling*

Phases	Minimum	Maximum	Mean
After exudation	4.7×10^2	7.0×10^3	3.7×10^3
After collection	1.2×10^3	3.5×10^3	2.4×10^3
At village market	1.0×10^3	5.0×10^3	3.0×10^3
At crops market	7.0×10^2	6.5×10^3	3.6×10^3
After 3months storage	2.3×10^3	5.0×10^4	2.6×10^4
After one-year storage	3.5×10^3	3.1×10^4	1.7×10^4
Kibbled gum	1.5×10	3.0×10	2.3×10
Mechanical powder	Nil	2.5×10	1.3×10
Spray-dried gum	2.5×10	8.0×10	5.3×10

*values for 6 samples

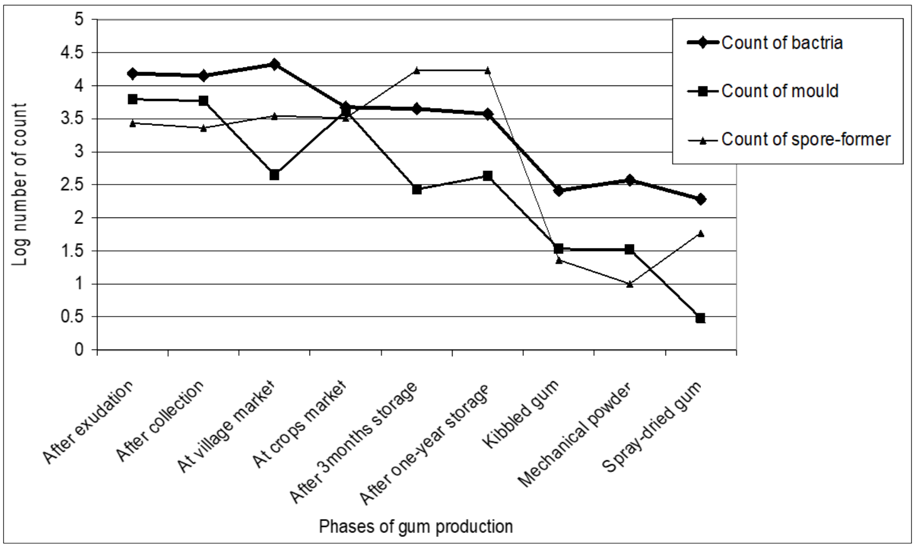

Fig 1: Variation between Count of bacteria, moulds and spore-formers bacteria

Staphylococcus aureus, *Salmonella spp* and coliform bacteria were detected at village market and at crops market but no *E. coli* was detected at these two points. No *Staphylococcus aureus* or *E. coli* was detected in samples after 3-month storage but *Salmonella spp* and coliform bacteria were detected. After one-year storage both *Staphylococcus aureus* and *Salmonella spp* were detected but no coliforms or *E. coli*. At processing of the kibbled and spray dried-gum no *Staphylococcus aureus*, *Salmonella spp*, coliform bacteria or *E. coli* were detected but in processing powdered gum *Salmonella spp* and coliform bacteria were detected (Table 4).

The 54 samples were examined for *Staphylococcus aureus*. Thirteen suspected isolates were obtained which were subjected to further confirmation tests. The following isolates were found eight may be *Staphylococcus aureus*. Checking for the presence of *Bacillus spp* was carried out on the 54 samples and twelve isolates were obtained. Further confirmation tests were conducted and the isolates were found to be as follows: Eleven out of twelve isolates turned out to be *Bacillus spp*. Out of the 54 samples sixteen isolates were also obtained. Further confirmation tests were conducted and the isolates were found to be as follows: Ten isolates may be *Klebsiella spp* while three were *Moraxella spp*, two *Enterobacter cloacae* and one was *Cedecea lipagei*. Twenty two *Salmonella spp* suspected colonies were isolated from 54 samples. Further confirmatory tests were conducted and the isolates were found to be as follows: Eleven may be *Salmonella spp* while the remaining five were found to be *Pseudomonas pickettii*, four were *Providencia spp*, one was *Serratia spp* and one was *Proteus spp*.

Identification of moulds and yeasts: All gum arabic samples at different stages of gum production showed that moulds were detected and isolates obtained were identified as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citreonigrum* and *Curvularia spp*. The present results was found to be in close agreement with the results obtained by Khalid (1984) [13], North East Wales Institute (NEWI, 1987) [18], Bokhary *et al.* (1983) [2], Anderson and Mc Dougall (1987) [1] and Idris (1989) [9].

Yeasts were not detected in any sample of gum arabic at different stages of gum production. This finding is in agreement with the results obtained by Idris (1989) [9], Osman (1998) [19] and Mohamed (2000) [17] and disagrees with the results reported by Anderson and Mc Dougall (1987) [1] who stated that natural gum carried a wide range of yeasts, and Ismail (2000) [10] who reported that total viable count of yeast ranged from 0.8×10^2 to 2.3×10^2 for crude gum arabic samples.

Table 4: Detection of some health indicator bacteria in gum arabic.

Gum Arabic samples	Number of Sampling point	*Staph. Aureus*	*Salmonella*	*Coliforms*	*E. coli*
After exudation	2.3.7.8.9.10	-ve	-ve	-ve	-ve
After collection	1	-ve	+ve	3.6/g	-ve
	3.6.10	-ve	+ve	-ve	-ve
	5	-ve	-ve	-ve	-ve
	11	-ve	-ve	2.4×10^3/g	-ve
At village market	1.9	-ve	-ve	-ve	-ve
	4.7	+ve	-ve	-ve	-ve
	5	+ve	+ve	-ve	-ve
	10	+ve	-ve	3.6/g	-ve
At crops market	2.9	-ve	+ve	-ve	-ve
	5	-ve	+ve	9.3×10/g	-ve
	7	+ve	+ve	4.3×10/g	-ve
	10	+ve	+ve	2.0×10/g	-ve
	14	-ve	-ve	3.6/g	-ve
After 3-months storage	2.6.7.8	-ve	-ve	-ve	-ve
	4	-ve	+ve	-ve	-ve
	10	-ve	-ve	2.1×10^2/g	-ve
After one-year storage	4.13	+ve	+ve	-ve	-ve
	5.9	-ve	+ve	-ve	-ve
	6.10	-ve	-ve	-ve	-ve
Kibbled gum	25	-ve	-ve	-ve	-ve
	29	-ve	-ve	-ve	-ve
	33.1	-ve	-ve	-ve	-ve
	36.4	-ve	-ve	-ve	-ve
Powdered gum	7	-ve	-ve	-ve	-ve
	29	-ve	-ve	-ve	-ve
	33	-ve	-ve	-ve	-ve
	36	-ve	-ve	-ve	-ve
	40.2	-ve	+ve	3.6/g	-ve
Spray-dried gum	43.5	-ve	-ve	-ve	-ve
	36	-ve	-ve	-ve	-ve
	27	-ve	-ve	-ve	-ve
	26.19	-ve	-ve	-ve	-ve

Conclusion and Recommendations

Conclusion

1. Hygienically, all gum samples collected aseptically from the trees were free of *Staphylococcus aureus*, *Salmonella spp* and Coliforms.
2. *Salmonella spp* and Coliforms were first detected on the gum samples collected by ferments while still in the field. *Staphylococcus aureus* was first detected in samples from the village market.
3. *Staphylococcus aureus*, *Salmonella spp* and coliform bacteria were detected at village market and at crops market but no *E. coli* were detected at these two points.
4. All gum arabic samples at different stages of gum production showed that moulds were detected and isolates obtained were identified as *Aspergillus niger*, *Aspergillus flavus*, *Penicillium citreonigrum* and *Curvularia spp*.
5. Yeasts were not detected in any sample of gum arabic at different stages of gum production.

Recommendations

1. More research is requirement to Evaluation the Microbiological of gum arabic samples were collected from others production Ares.
2. Need research possessing to degrease the Microbiological in gum arabic for all handling chin

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