DECONTAMINATION PROCEDURES FOR MICROBIALLY CONTAMINATED LOCAL STARCHES

Igwilo, C., * Akpan, U.E., Alo, B., Nasipuri, R.
And Rabiu, O. School of Pharmacy, College of Medicine, Idi-Araba, University of Lagos.
*correspondence

Abstract

Corn and cassava starches, isolated from local corn grains and cassava tubers and predried to a moisture content of less than 2.0% were exposed to microbial contamination. These contaminated starches were then exposed to various sterilization processes, which include dry heat, and ethylene oxide gas at specific conditions, for different time periods.

These samples were subsequently subjected to U.S.P. (1985) sterility and gross contamination tests from the various results, sterilization by ethylene oxide gas appeared to be the most effective method in reducing bacterial count, followed by the dry heat method.

Samples of starch exposed to the sterilization processes, and stored for 3 months at room temperature showed neither visible nor microscopical degradative characteristic.

Though absolute sterility is not a pre-requisite to the use of pharmaceutical grade starch for manufacturing, nevertheless, a high microbial count of non-pathogenic microbes is undesirable for stability reasons. Thus, from the results of this project, it is possible to considerably reduce the high microbial contamination of most locally processed starches.

Introduction

Starch is an important adjuvant in the formulation and production of tablets. It is used for such functions as filling (diluent), binding, disintegration, lubrication and inhibition of dye imigration in tabletting. Its relative inertness, abundance and cheapness favour its choice from a list of excipients available to a tablet formulator.

Several studies have been carried out to evaluate the suitability of the locally manufactured pharmaceutical grade starch obtained from corn grains, cassava, yam and cocoyam tubers and plantain, for their use as tablet excipient. Pioneering work was done by Mital and Ocran in 1968. They evaluated the disintegrant properties of both cassava and yam starches. Nasipuri, in 1975) also conducted a comparative study of the binding and disintegration properties of cassava and B.P. potato starches on sulphathiazole and promethiazine HCI tablets. Jaiyeoba and Opakunle, in 1978 prepared granules of cassava and yam, starches which they referred to as modified starches. All these studies mentioned above and many others done on locally manufactured pharmaceutical grade starch gave favourable results which thus strongly recommend the use of these locally processed starches as tablet excipient. However investigation revealed that most of the pharmaceutical industries do not use any of these extensively investigated starches, often as a result of their high microbial content. The observation thus supports the findings of Jaiyeoba (1986) who implicated starch as the most prominent highly contaminated formulation excipient.

Although some level of non-pathogenic microbial content is allowed in non-sterile medication but it is highly desirable that products should have microbial contents controlled at a low level both in terms of total viable organisms, for stability reasons, with total absence of undesirable potential pathogens.

Therefore the objective of this work is to establish an effective microbial decontamination procedure for starch samples with high level of non-pathogenic microorganisms with the aim of alleviating some of the problems associated with the use of locally produced starch in our pharmaceutical industries. This is hoped to contribute positively towards achieving the much desired self sufficiency in drug production in our sub-region.

Material

Sabourand - Dextrose Agar (SDA - used for culturing moulds), Tryptone - soya agar (TSA - used for culturing bacteria). Tryptone - soya broth (TSB - use for culturing bacteria) and Bacto - peptone (sterile diluent) were obtained from Difco Laboratories U.S.A. and were used as received. Fluid Thoglycollate Medium (U.S.P) was obtained from Becton, Dickinson and Company U.S.A.

Cassava and Corn starches obtained from Mannihot utilissima and Zeamays were prepared in the laboratory.

Methods

(a) Preparation of Corn Starch

Dried, white grains of Zea mays were soaked in adequate quantity of distilled water maintained at 48°c for 24 hours. The softened corn grains were expressed and the slurry passed through a 200 um mesh sieve. The starch milk was allowed to settle and washed several times with distilled water until the starch was completely free of gluten. The clean starch was then dried in an hot air Oven 650 for 4 hours. The dried starch was reduced to a fine powder and sieved through a 200 um mesh sieve.

(b) preparation of Cassava Starch

Cassava starch was isolated in the Laboratory according to the method adopted by Mital and Ocran (1968).

(c) Determination of Moisture Content

The moisture content of starch powder obtained from Zea Mays and Mainhot utilissima were determined using the Ohaus moisture determination balance.

(d) Microscopic Examination

0.5% w/v of solution of starch samples were microcopically examined at x 40 magnification before and immediately after decontamination processes and also 3 months later, after decontamination procedure. This was to find out, the probable adverse effect the decontamination procedure could have on the shapes and sizes of the starch grains.

(e) Microbial Decontamination Exercise

The methods adopted were:

(i) Exposure to Dry Heat

10 gm sample each of both corn and cassava starches were exposed to 65°, 80° and 98° Lot air oven (Hotpack Tru-Temp Oven, USA) temperature for 2,4 and 6 hours. The range of 65 - 98°c has

been chosen because starch cannot tolerate higher temperature values at the exposure time periods considered.

The samples were aseptically unload into a sterile chamber, after exposure, and subjected to the U.S.P. aerobic viable count and sterility tests.

(ii) Exposure to Ethylene Oxide Gas

10 gms of each starch sample was exposed to the ethylene Oxide gas, (AMSCO Sterilizer U.S.A.) maintained at 15 pounds pressure, 30% humidity and 50°c gas chamber temperature for varying time periods

The samples were subjected to the official (U.S.P.) test after specific exposure periods.

(f) Total Aerobic Count (Plate Method, USP 1985)

10 gms of each of the starch sample before and after the decontamination procedure was weighed into 90 ml of sterile phosphate buffer (ph 7.2).

A two.step 10 fold serial dilution of the above solution was mac by using 1 ml aliquots per 9 ml of sterile phosphate buffer in each step.

1 ml each of the final dilution (containing 0.001g of Starch) was added to two different duplicate petri dishes, one pair containing 20 ml of TSA and the other, 20 ml of SDA. The TSA and SDA plates were incubated at 37°c and 25°c respectively for 5 days. Following incubation, the plates were examined for growth and the number of the colonies counted. The result were expressed as the average number of microorganism per gram of the sample.

(g) U.S.P. Sterility Tests (Membrane Filteration Method)

Sterility test was performed with the U.S.P. Sterility kits fitted with 0.45 u membrane filter. 0.1% peptone water was used as the sterile diluent and TSB and FTM employed as the nutrient media. The test was performed under a Laminar flow of sterile air.

Only those starch samples that showed no aerobic microbial colony count after decontamination procedure were subjected to the U.S.P. Sterility Tests.

The sterilized starch sample was interpreted to have passed the sterility test, if no growth is noticed within seven days of culture and incubation.

Results and Discussion

The moisture content of corn starch was 1.85% and cassava starch 1.65%. They were maintained at this low moisture content values, inorder to prevent gelatinization, during exposure to high dry heat temperature. The corn and cassava starch grains gave identical characteristic shapes and sizes, both before and after decontamination exercises.

Fig 1 shows the effect of temperature (Dry heat) on microbial growth. The microbial growth decreases as the temperature increases. This was probably due to oxidation of the cell content. The former were more susceptible to dry heat than bacterial cell as the former population decreased at a faster rate than bacterial. This was probably due to the differences in their cell morphologies. Charring of the starch powder was observed to occur at 80°c and 98°c after specific exposure periods which was evident by the some brownish black specks. This was probably due to the denaturation of the starch grains, and is one of the major disadvantages of dry heat sterilization methods for powders.

Fig. 2 shows the effect of ethylene oxide gas on the microbial growth after different exposure periods. The microbial colony counts were found to decrease progressively as the exposure time was increased. Corn Starch and Cassava Starch exhibited no microbial growth after 8 hours and 10 hours exposure to ethylene oxide respectively. The observed antimicrobial action is due to the ability of ethylene oxide to alkylate-SH -OH-COOH and - NH_2 groups in enzymes, proteins and nucleic acids: for example,

(i) Protein $-NH_2 + C_2H_4O$ - Protein - NH ($_2H_4OH$)

(ii) Protein - SH + C_2H_4O - Protein - S-C_2H_4OH)

Although it has been shown above that ethylene oxide is effective in reducing mircobial count of starch, but the Medicines Commission Report of 1973 on the prevention of Microbial contamination of medicinal products, has however warned on the potential risks of chemical incompatibilities and production of Toxic residues and to its inefficiency against organisms occluded in crystal or otherwise protected from the gas.

Table 1 shows the results of the USP sterility tests performed on those starch samples that exhibited no microbial growth after exposure to ethylene Oxide gas. Sterilization of starch samples was only achieved after 62 hours exposure to ethylene Oxide gas.

Conclusion

A comparative study of the different sterilization procedures that could be used to reduce the microbial population of locally processed pharmaceutical grade starch with a high level of non-pathogenic microorganism have been made.

Dry heat was found to be reasonably effective at 80° and 90°c for 2 hours respectively. Drying at 80°c for 2 hours (since charring of starch is less likely to occur at this temperature compared with 98°c) may be included in the manufacturing procedure for the local production of pharmaceutical grade starch, coupled with a high level of hygiene so that the initial level of contamination is reduced considerably.

Ethylene Oxide gas was found to be most effective method of reducing microbial count from the results of the experiment. However, the exorbitant operational cost and the risk factors highlighted by the Medicine products (1973), will not favour the routine use of Ethylene Oxide gas in the processing of pharmaceutical grade starch from local sources.

This study has shown that locally processed starches which hitherto have been rejected for pharmaceutical use on the ground of heavy microbial contamination could be rendered useful through the various decontamination techniques which might result from usage of ethylene oxide, the dry heat process seems the most probable method of choice for starches with very low moisture content.

References

1. Mitah, H.C. & Ocran, J (1968). Pharm. Acta Helv. 43, 493.
2. Naispuri, R.N. (1945) J. Med, Pharm. Mark 4, 17.
3. Jaiyeoba, K.T. and Opakunle, W.O. (1978)
4. Jaiyeoba, K.T. (1988) in Pharmaceutically Raw materials sourcing in Nigeria.

   Proceedings of 1st symposium on Nigeria Pharmaceutical Industries Ibadan, 14.