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EXTRACTION AND CHARACTERIZATION OF GELATIN FROM COW BONES: USING SUBS ITUTE DEMINERATIZING AGENT (H_2SO_4)

E. M. OKONKWO, C. S. EZEANYANASO, O. OFOEGBU, E. ALI AND N. T. IYAMU

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ABSTRACT

Gelatin has always been obtained from animal bones by demineralization with HCl followed by alkali treatment. In this work gelatin was obtained from bones by demineralization with H_2SO_4 and comparison with that obtained from HCl route was done. The products were characterized by determining the pH at 10% concentration, viscosity, colour, taste, solubility in solvents, particle size, smell, moisture (%) and ash (%). The results (pH=6.7 moisture=10.5±1.5 and % Ash 1.8 ± 0. 4) show that H_2SO_4 can be used in place of HCl in the production of gelatin from bones. This will reduce the overall production cost of manufacturing gelatin from bones considering the relative higher cost of HCl. The results of the viscosity, gel strength, pH, moisture content and antimicrobial tests: viscosity =55, gel strength = 300g Bloom, pH = 6.7, % moisture content = 10.5± 1.5, when compared to standards, support the possible utilization of this grade of gelatin for industrial and pharmaceutical applications.

KEYWORDS: Cow bone, Defattening, Decalcification, Liming, Gelatin.

INTRODUCTION

Gelatin a pure collagen protein from animal raw materials is obtained from animal hides and skin, tissue and bones (Boque 1922). Gelatin is an easily digested pure protein food though nutritionally incomplete due to the deficiency in amino acids (Brown et.al. 1990). There are two basic grades of gelatin, an acid processed type known as (type A) and an alkali processed type known as type B (Hill 1965).

Type A gelatin has been made mostly from pork skin yielding grease as a marketable byproduct while type B is made mostly from bones and it yields dibasic calcium phosphate as an important byproduct (Ward A.G. 1958).

About 65% of the worldwide produced gelatin comes from hide splits, connective tissues and the bones of cattle, though in Australia, South Africa and New Zealand sheep are also used (Korpft et.al. 1972-1982). However, the quality of gelatin is partly determined by the source of supply, which in Europe traditionally is mainly from pigs (for food and medication). In Germany, only slaughter wastes from animals which are released for human consumption are used for the production of gelatin (for food and medication)(Pattison et.al. 1962). The fear and uncertainty from many consumers of gelatin is as consequence of BSE (Bovine Serum) contamination crisis in gelatin containing products, because till date attack of BSE is only diagnosed at a very late stage (mostly at the completion of the full life cycle) and most infected animals are not identified before slaughter. In order to overcome this, gelatin without BSE-infectivity, can only be produced from healthy animals. It is known that some of BSE–infected animal parts are far less infectious than others (Rinder 1995) and bone is one very advantaged part among others. The ability to detect BSE by any sensitive chemical/instrument test, in anatomical parts cannot be comfortably ascertained, so the use of less susceptible parts in the production of gelatin is encouraged.

No pure bones (due to compositional make-up of bones), have been employed in gelatin production consequently the product has always been graded type B as a result of application area and product characteristics (Korpft et.al. 1972-1982). However, type B gelatin is expected to be produced from this work and this type of gelatin has an exciting wide field of applications for quality enhancement of innumerable foodstuff and medicaments, serves as supplementary sources of protein carrier material, stabilizer and emulsifier. It is also used in flavour enhancement, as a clarifying agent in brewery industries to remove or eliminate turbidity and particle suspension in products such as beer, wine, fruit juices etc.

In pharmaceutical industries, gelatin is used in soft or hard capacities for binding tablets, capsule coats, sponges for treating wounds. They are component of vitamin formulations and cosmetics. They are also used in adhesive industry due to their gelling strength.

One of the two primary aims of this work is to possibly refine the production process conversion of bones to gelatin in order to obtain a "Type A" grade product. Presently, there is no known industry producing gelatin in Nigeria. Virtually all gelatin been used are imported (Taylor, et.al. 1994) and the success of the project will optimize the economic value of discards of bones by increasing the number of products obtained from it.

EXPERIMENTAL

Materials

The bones were obtained from local abattoir in Zaria. H_2SO_4, HCl, NaOH and n-hexane were obtained from BDH, Poole, UK., $Ca(OH)_2$ was obtained from the hydrated lime plant of the National Research Institute for Chemical Technology (NARICT) Zaria.

Equipment

Soxhlet extractor, heating mantle, distillation unit, beakers, muslin cloth, bone crusher, Edwards high pressure vacuum pump, Solvent recovery unit and oven drier.

Method

(1) **Defattening/Degreasing of Bones**

50 g of bones were reduced to small sizes of 3 inches approximately and put into the soxhlet extractor with 250 mls of n-hexane added. Extraction was carried out for a minimum period of 6 hours. The degreased bones were dried in an oven at a temperature of about 70°C

E. M. Okonkwo, National Research Institute for Chemical Technology, P. M. B. 1052, Zaria-Kaduna, Nigeria
C. S. Ezeanyanaso, National Research Institute for Chemical Technology, P. M. B. 1052, Zaria-Kaduna, Nigeria
O. OFoegbu, National Research Institute for Chemical Technology , P. M. B 1052. Zaria-Kaduna, Nigeria.
E. Ali, National Research Institute for Chemical Technology, P. M. B.1052, Zaria-Kaduna, Nigeria
N. T. Iyamu, National Research Institute for Chemical Technology, P. M. B. 1052, Zaria-Kaduna, Nigeria.

(2) **Demineralization**

25 g of defattened bones were soaked in a container containing dilute solution of H_2SO_4 at a concentration of about 0.15mol/dm^3 for a period of 4 days with a daily periodic agitation of the content. Second set of the defatted bones were soaked in a container containing dilute solution of HCl with a concentration of about 0.15mol/dm^3 for a period of 5 days with a daily periodic agitation of the entire content. This dissolved the calcium sulphate, calcium phosphate and other mineral matter leaving the organic matter. Afterwards the acids are washed off thoroughly with distilled water. This ossein (demineralized bone) obtained was then stored for the liming processes.

(2) **Liming and Extraction**

The ossein obtained was divided into two sets. The first set of ossein were put in a container and soaked in alkaline solution (NaOH in water, pH between 9-11). This was soaked for a period of 14 days with a daily agitation of the contents (30minutes). The second set of ossein were put in a container and soaked in lime water (Ca $(OH)_2$ in water). This was soaked for a period of 30 days with daily agitation of the contents (30minutes). The resultant materials were then washed with distilled water and with slightly acidulated water (pH 5.5) to adjust the pH to between 6.5-7.0.

Series of extractions were carried out starting from 50°C to 100°C. The extracted gelatin were then filtered off, concentrated, oven dried and ground into powder.

RESULTS

The results of the characterization of the products obtained and comparisms made with imported gelatin are presented as table 1.

S/No	CHARACTERISTICS	VALUE		
		Standard	H_2SO_4	HCl
1	Colour	Light brown	Milky white	Light brown
2	Particle Size	Coarse to fine	Fine	Coarse
3	pH (10% of Product in Water)	4.5 to 7	6.7	6.6
4	Taste	Slight taste of a Seasoning	Tasteless	Slight taste of a seasoning
5	Smell	No noticeable smell	No noticeable smell	Faint smell of seasoning
6	Moisture content (%)	10 ± 0.9	11.5 ± 0.5	13.0 ± 1.5
7	Ash content (%)	1.8 ± 0.5	1.8 ± 0.5	1.9 ± 0.3
8	Yield (%)	45 to 50	45±0.2	46±0.2
9	Solubility in:			
	Water	Soluble in hot water	Soluble	Soluble
	Ethanol	Slightly Soluble	Slightly	Slightly
	Carbon Tetrachloride	Not Soluble	Not soluble	Not soluble
10	Setting Point (Temp. at which 10% aqueous gelatin solution gels)	35°C	35°C	35°C
11	Viscosity (mp)	47	46	49
12	Gel Strength ("Bloom" value)	90 to 300	242	245
13	Isoelectric Point	4.8 to 9.4	6.5	6.2
14	Decomposition Temp	From 100°C	120°C	121°C
15	Combustion Temp	From 500°C	600°C	620°C
16	Swell-ability at 30-35°C	5 – 10 x its wt of water and form gels	7 x its wt. of water and forms gels	6 x its wt .of water and forms gels

Table 1: Results of the physico-chemical analysis of produced gelatin samples and comparism with imported sample. (Values for the Standard are courtesy of Vyse Gelatin Company Inc. 2002 Material Safety Data Sheet)

DISCUSSION

The result of the characterization show that for which ever route followed, the final gelatin properties does not change or affected by the use of either of the mineral acids. Technically speaking there is no significant difference between type A and B grades of gelatin when the conversion of asparagines and glutamine residue to their respective acids and high viscosities is inconsequential (Eeastoe, J.E. et al 1958). This is because the major difference in grade is borne from the fact that acid pre-treatment does not convert these substances while the alkaline pre-treatment does (Cole C.G.B. et al 2002). Where an exhaustive purification process is carried out gelatin from any source can be obtained as type

"A" grade and used for any kind of industrial application, and when proper acid neutralization is carried out, the H_2SO_4, treated collagen material can be employed for pharmaceutical purposes. This is preempted by the result of the microbial analysis from which deduction is made based on the ability of microorganism to feed on the gelatin samples (Schwimmer M. el al 1957). This makes the possible utilization of these samples (not minding the process treatment steps that is followed) in the pharmaceutical industry.

The cheaper cost of H_2SO_4 when compared to that of HCl(the former is locally produced by some indigenous companies like Duree chemicals plc Kaduna, provides an overall product cheapness, which will boost the profit margin for the entrepreneur who chooses to employ the use of H_2SO_4 as his decalcification agent. The residue, which is primarily dicalcuim phosphate and bone meal, will also serve as good sources of phosphate fertilizer or soil conditioner. This integration system ensures that no part of the raw material feedstock is wasted and there is an enhanced product output capacity with consequent profit maximization.

The defattening solvent, n-hexane is recovered from the process system with less than 2% loss and recycled with out any form of environmental hazard. The fat can be employed in certain industrial applications (soap making, animal feed, etc.) and is another source of revenue for the entrepreneur.

CONCLUSION

It is economically viable to produce gelatin from bones using H_2SO_4 as demineralizing agent.

The characteristic properties of the product obtained from H_2SO_4 process does not differ significantly from that of a conventional HCl process.

The gelatin product from H_2SO_4 process can be used in the pharmaceutical industry (with exhaustive sterilization) as well as other areas of application like food, photography, cosmetics, coating and sizing of paper protective colloidal applications. The gelatin can also be used to produce matches adhesive coated abrasives etc.

The setting up of such a plant in the country will cut down our dependence on imported gelatin, while creating jobs and will also reduce the cost of products that use gelatin in their manufacturing process.

The production of value added products like the dicalcium phosphate and bone meal will create a raw material source for the organic fertilizer plant and ensure a perfect zero waste process system (on-going project work).

H_2SO_4 which is locally and cheaply obtained from our local market(as earlier mentioned in the text), provides a more economical substitute for the production of high grade gelatin material than HCl, which is basically imported costlier

The H_2SO_4 substitution ensures a 100% locally sourced raw material technical processing of gelatin because the acid is locally produced, the bones locally sourced and the alkali obtained from a locally based company.

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