CASEIN EXTRACTION AND FORMATION OF BIODEGRADABLE AEROGEL

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Abstract—**Biodegradable plastics unlike conventional petroleum based plastics are not decomposed by combustion and can be degraded in favourable environmental conditions. The scarcity of space for disposal of plastic waste and growing environmental concerns for non-biodegradable synthetic plastics have fuelled research towards development of eco-friendly biopolymer materials. It is very difficult to reduce the consumption of plastic products due to their versatile properties, but it is possible to replace petroleum-based plastics with alternative materials that have polymer-like properties and that degrade after being discarded. In this paper we present a concept of producing a biodegradable polymer from casein which is protein extracted from milk. Casein extracted from various milk samples is compared for its yield and the sample with maximum yield is then blended with the plasticizers to form a biodegradable polymer and can be used for various low density polymer applications. The test of Aerogel absorbing water and is biodegradable is also done. Similarly polymer formation is confirmed by FT-IR analysis of the product formed.**

Keywords**—** casein, clay, aerogel, biodegradable, polymer

I. INTRODUCTION

Growing environmental concerns have led to increased emphasis on research and development of biodegradable materials based on renewable biological resources for biomedical and industrial applications. The naturally occurring biopolymers such as collagen or gelatin, chitin or chitosan (1), zein, soy protein, wheat gluten ([2],[3]), starch (4), cellulose, pectin (5) and so on are used in the production of composite films and coatings ([6],[7]). Excellent bioplastics may be developed from biologically derived polymers by proper blending and processing techniques(5). Their functionalities can also be tailored by plasticizers, cross-linkers, and other additives. The paper enlightens the conversion of milk protein casein as a biodegradable polymer ([8], [9]) when blended with plasticizers and cross linking agents.

II. CASEIN

Casein is a naturally occurring macromolecule that accounts for approximately 80% of the protein content of cow's milk; it is a phosphoprotein that can be separated into various electrophoretic fractions, such as αs-casein, κ-casein, β-casein, and γ-casein in which each constituent differs in primary, secondary, and tertiary structure, amino acid composition, and molecular weight ([10]-[12]) Its structure comprises of presence of amino acids. Amino acids have a variety of chemically reactive groups like phenolic hydroxy groups, presence of peptide bonds. Casein also includes amino groups, ketones and hydrazine groups. The caseins are suspended in milk in a complex called a micelle. The caseins have a relatively random, open structure due to the amino acid composition (high protein content). The high phosphate content of the casein family allows it to associate with.calcium and form calcium phosphate salts. But casein is not very strong, and water can wash it away. To beef up casein, and boost its resistance to water, the scientists blended in a small amount of clay and reactive molecule formaldehyde, which links casein's protein molecules together.

III. PRESENCE OF CASEIN IN MILK

Milk contains 3.3% total protein. Milk proteins contain all 9 essential amino acids required by humans. Milk proteins are synthesized in the mammary gland, but 60% of the amino acids used to build the proteins are obtained from the cow's diet. Total milk protein content and amino acid composition varies with cow breed and individual animal genetics. There are 2 major categories of milk protein that are broadly defined by their chemical composition and physical properties. The casein family contains phosphorus and will coagulate or precipitate at pH 4.6. The serum (whey) proteins do not contain phosphorus, and these proteins remain in solution in milk at pH 4.6. The principle of coagulation, or curd formation, at reduced pH is the basis for cheese curd formation. In cow's milk, approximately 82% of milk protein is casein and the remaining 18% is serum, or whey protein.

IV. EXPERIMENTAL WORK

The experiment for extraction of casein was carried out for three milk samples with varying fat content. The results were compared based upon the yield obtained after the extraction in which precipitation was caused using glacial acetic acid manufactured by High Purity Lab Chemicals Pvt. Ltd. Mumbai; and was used without any further modification. Casein was extracted from SAGAR Skimmed Milk Powder Spray Dried of non-fat protein rich milk supplied by Gujarat Co-operative Milk Marketing Federation Ltd. and AMUL milk samples of different fat content.

TABLE I

NUTRITIONAL INFORMATION OF MILK SAMPLES

Type of milk sample	**Fat**	**Protein**
A	1g per 100g of milk powder	35g per 100g of milk powder
B	4.5 g per 100ml	2.89 g per 100ml
C	3.5 g per 100ml	3.2 g per 100ml

The milk samples which were used had difference in the amount of fat content and protein content are:

Sample A: non-fat milk powder,

Sample B: Amul Shakti millk,

Sample C: Amul Cow milk

The nutritional difference in the taken samples are compared above in Table 1.

V. CASEIN PREPARATION

In the laboratory casein extraction from non-fat milk powder was carried out. 80 ml of distilled water after heating at a temperature of 55 °C is then mixed with 20 gram of non-fat milk powder.The mixture is then stirred in which 10 ml of acetic acid is added drop by drop. This results in the formation of precipitates of casein which is then washed 2 or 3 times using water after decanting off the filtrate which is then rubbed with sufficient amount of 0.1% sodium hydroxide solution and then the resulting solution is filtered using a cloth as shown in figure 1. Precipitates termed as casein, are then treated with 10 ml of 1:1 alcohol and ether solution is added to remove remaining fat content if present.

Fig. 1 Casein product in powdered form

A Process yield

Weight of milk powder used initially = 20 gram
Weight of glacial acetic acid = 1.015 gram
Weight of product obtained = 16.74 gram
$ProcessYield = dryweightofproductobtainedweightofmilkpowder + glacialaceticacid \times 100$
= 16.74(20+1.015)
= 79.657 %
Thus a process yield of 79.657 % is obtained for the production of casein.

On a similar basis the yield calculated for other samples is listed below in Table 2.

TABLE 2

CALCULATED PROCESS YIELD FOR VARIOUS MILK SAMPLES

Milk Sample	**Calculated Yield (in percentage)**
A	79.657
B	27.683
C	37.452

As the maximum yield was obtained for nonfat milk powder sample because of minimal fat proportion it was further checked with confirmatory tests of the functional group and was used for further polymer aerogel formation.

B *Test results for the experiment*

Since casein structure is composed of variety of functional groups like carbonyl groups, phenolic hydroxyl group, amino acids, peptide bonds it can be confirmed using confirmatory tests for various functional groups present in it.

- Confirmatory Test for presence of Protein: Xanthoproteic test: confirmation by dark yellow colour.
- Biuret Test: Confirmation of peptides bond by purple colour

While confirmatory tests for the detection of carbonyl groups, presence of amino acids were also carried out and positive results were obtained. Thus, the precipitates formed were confirmed as casein.

VI. AEROGEL FORMATION USING EXTRACTED CASEIN

Casein was solubilised and emulsified in an aqueous solution of 0.05 N sodium hydroxide by heating the mixture at 100 °C for 2 hours. Once the casein formed a thick emulsion with aqueous NaOH, it was blended with formaldehyde and then again heated for 15 min at 100°C. The mixture is then air dried in a flat tray at ambient temperature conditions (around 25 °C). A thin film around 2-3mm thickness is then formed. The formed aerogel film is then kept for heat treatment for 24 hours at a high temperature around 130°C. The heat-treated films were then exposed to relative humidity conditions of room air at room temperature for examination of the effect of moisture on mechanical properties of these films. The formed film is shown in figure 2.

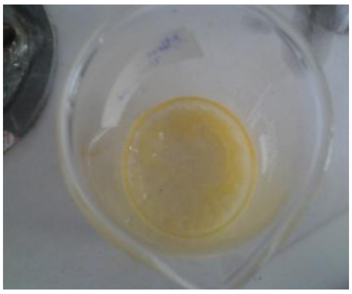

Fig. 2 Casein Aerogel polymer film

VII. WATER ABSORPTION (SWELLING OF GEL)

Aerogel absorbs 8 times of its own weight in water and hydrogels undergo shrink to approximately one-fourth of their starting size.Numerous cycles of above reversals and no breakdown in structure or performance shows increase in its weight upto certain limit.Weight gains in the aerogel composites is calculated using

$$Water\ absorption\ (\%) = \frac{Wwet - Wdry}{Wdry} \ * 100\ \%$$

Sample immersed in water and increase in weight was calculated for a duration of every 30 minutes. The readings are shown below in table 3.

TABLE 3

WEIGHT OF GEL WITH RESPECT TO TIME

Time (Min)	Weight(gm)
0	0.29
30	0.31
60	0.33
90	0.35
120	0.38
150	0.42
180	0.45

For W_{wet} = 0.31g
W_{dry} = 0.29g
Water absorbed = $[(W_{wet} - W_{dry})/ W_{dry}]* 100$ = 6.89 % . Using the same formula, results are calculated and represented in table 4.

TABLE 4

PERCENTAGE INCREASE IN WEIGHT WITH RESPECT TO TIME.

Time (Min)	Increase in Weight(%)
0	-
30	6.89
60	13.79
90	20.68
120	31.03
150	44.89
180	55.17

Improving water Resistance

Swelling characteristic is strongly influenced by the cross-linked microstructure of the polymers. Casein film made from aqueous NaOH solution swelled to about 55wt % and then collapsed and dissolved rapidly.Water resistance can be improved by clay blending, due to the strong hydrogen bonding and covalent interactions of casein proteins with clay resulting in cross-linked protein polymer .Swelling in water ofthe sample can be decreased by blending it with plasticizers and clay which also improves its mechanical strength.

VII. Results and Discussions

The casein extraction was carried out for milk samples with different fat content. The above process of casein formation results in the formation of a product which is in lumps due to separation as precipitates. The lumps are then grinded andreduced in the powdered form. The conversion of casein from milk powder of non-fat protein rich milk sample gave the highest yield out of the three samples. The casein is a protein molecule with elastic properties, high mechanical strength and also insoluble in water. Due to such properties its suitability to be used as a polymer increases. To improve upon the properties of casein it is blended with formaldehyde as a cross linking agent, which combines with the lysine residue for the aerogel formation as per the Maillard reaction. The reaction in figure 3 shows a reaction for glyceraledehyde molecule which has a similar chemistry of bonding as that of formaldehyde and casein. Lysine is the basic amino group which is present in proteins.

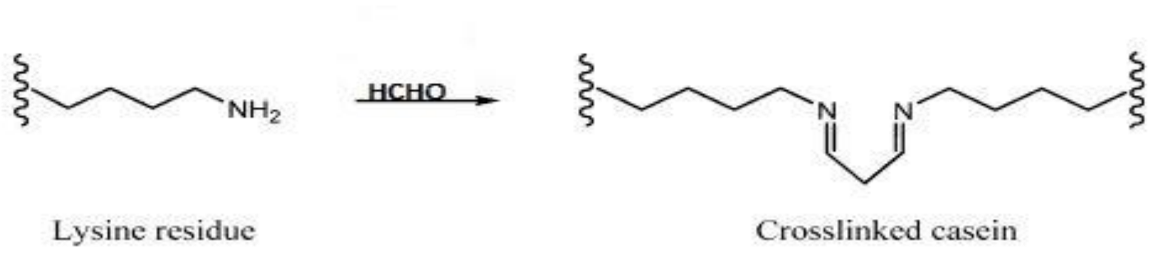

Fig.3 Cross linking mechanism by Maillard reaction [1]

FT-IR (Fourier TranformInfra Red) Spectral analysis was done of the casein extracted and the aerogel formed using Shimadzu FT-IR spectrophotometer. Characteristic peaks of casein were located around 770, 1515, 1639, and 2350 cm^{-1} which corresponds to C=O stretching, both C-N stretching and N-H bending, C-H deformation, and N-H deformation, respectively. Comparison of both spectra also indicated the formation of a polymer.

The aerogels exhibit useful rates of biodegradation and these rates are enhanced by chemical crosslinking of the casein polymer. Aerogel composite was kept for degradation in soil for a span of 18 days

- Initial weight = 0.20g
- Final weight after 18 days = 0.16g

A 20% reduction was observed in a span of 18 days, so it can be concluded that the polymer formed is biodegradable in nature.The formedaerogel can serve as a viable substitute for conventional non-biodegradable polymers used for low density, high temperature applications. These materials may have several potential applications including the fabricationof bioscaffolds, foams, contact lenses, drug delivery capsules, and numerous other technological applications

VIII. Scope for further improvements in properties of aerogel composite

The aerogels exhibit useful rates of biodegradation, rates can be increased by chemical crosslinking of the casein polymer, with the attendant changes in its protein structure ([8]).Addition of clay to these polymers has gained interest as they have been shown to act as crosslinking agents, increasing the mechanical properties of the composites ([13],[14]). These improvements in properties are primarily driven by good dispersion and/or the ability of clay to exfoliate in the polymer.An alternative route for the incorporation of clays into organic polymers could be topreform clay matrices that do not require exfoliation and then fill or impregnate these clay structures with polymer (melt orsolution) or monomers (which then could be polymerized in situ)range of polymeric matrixes and from the absence of thermodynamic and kinetic barriers present when polymerisation reactions are conducted within the more traditional swelled clays ([15]-[16]). By using a clay aerogel as the inorganic filler, no exfoliation of clay sheets would be required, and polymerization reactions could potentially proceed in a normal manner. So this casein aerogel can be converted into casein aerogel and clay composite which has widespread applications.

Acknowledgement

The authors thank Neha Tripathi and Sweta Bosmia for performing the experiments and Chemical Engineering Department of Institute of Technology, Nirma University, Ahmedabad for providing the requirements and laboratory facilities for the experiment work.

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