Eggshell Membrane Separation Methods-Waste to Wealth-A Scoping Review

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Abstract
Eggshell is the most abundant agricultural (hatchery) and industrial (confectionary) natural waste approximately reaching up to 50000 tons per year and is a threat to the environment and the public. Eggshell membrane and shell waste can be used for biomedical and industrial applications as value-added products. Hence, a scoping review was planned to understand the present scenario and knowledge gap; it also explored eggshell membrane separation methods available commercially for the routine utility to understand the knowledge gap. A systematic search was performed in PubMed, Scopus, and ProQuest databases using Eggshell/Eggshells/Egg shell, Membrane, Separation/separator as keywords with and/or as Boolean operators within the field of title, abstract, keywords. The search was done from the period of inception (as per the database) till 4th October 2021 and a total of seven full-text articles were included in the review. The methods mentioned in the literature were Microwave treatment, Acid Dissolution, Dissolved Air Floatation, using proteolytic enzymes, and grinding followed by mechanical agitation. This research concludes that no equipment is commercially available. Hence there is a need for technology development as the majority of publications mentioned manual peeling, Microwave treatment, acid dissolution, dissolved air flotation, grinding, and mechanical stirring as the methods at laboratory scale.

Introduction
Eggshell is an abundant natural waste arising from agricultural (hatchery) and industrial (confectionary) waste. It is mostly discarded as a landfill or burned that contributes to different types of pollution like soil, water and air. According to FAOSTAT (Food and Agricultural Organization Corporate Statistical Database) production data (2020), India ranks third in egg production across the globe (Echap07.Pdf, n.d.). The production of eggs in the country has been almost doubled. This was increased to 114.38 billion from 78.48 billion in the year 2014-15 to 2019-20 (Seeking Comments on National Action Plan- Poultry- 2022 by 12-12-2017.Pdf, n.d.). The shell weighs about 11% of the total mass of the egg and its chemical composition consists of calcite that ranges from 94–97% magnesium carbonate, calcium phosphate, and 3–4.5% organic matter (John-Jaja et al., 2016; Pliya & Cree, 2015; Siva Rama Krishna et al., 2007). In addition, small amounts of other elements have also been detected that includes MgO (0.83%), Al2O3 (0.15%), SO3 (0.66%), K2O (0.08%), P2O5 (0.43%), Cl2O2 (0.06%), SrO (0.04%) and SiO2 (0.07%) (Boronat et al., 2015).

However, the percentage of composition slightly varies. The eggshell waste generated globally contributes to approximately 50000 tons per year and is a threat to the environment and the public. Usually, the eggshell waste is disposed of mostly as a fertilizer (26.6%), ingredient for animal feed (21.1%), into...
The concept of landfills is not preferred because the shells along with the attached membrane attract vermin. Eggshell without separating the membrane is of no use. So, many research groups have been working on the separation of the membrane from the eggshell for various applications at the lab scale.

The use of eggshell has been used in various biomedical applications like tissue engineering (Sah & Rath, 2016), osteoarthritis (Ruff et al., 2009), anti-inflammatory (DeVore et al., 2007), hypertension (JP2008007419A.Pdf, n.d.), gastrointestinal disorders, wound dressing and healing (Ahmed, Suso, et al., 2019; Li et al., 2016), therapeutics (Long et al., 2008), nutraceuticals (Ahmed, Kulshreshtha, et al., 2019), and cosmetics (Marimuthu et al., 2020) along with several industrial applications (King’ori, 2011) at the lab scale (Ahmed, Wu, et al., 2021). But the methods used for separation are not universal and not available for the common man (small, medium, and large-scale hatchery and confectionery industry) as a waste management tool. This waste to wealth research projects and products would produce both economic and environmental advantages if these are available economically in the market.

The concept of separation of membrane from eggshell made the authors curious to systematically search various databases, technologies, or methods that are present for separation of the membrane. Hence, this scoping review aims to explore eggshell membrane separation methods/techniques and commercially available equipment for the use of the common man.

Materials and Methods

The purpose of this scoping review is to have an overview of the available evidence by summarizing the existing available literature related to the research area. There is a clear mandatory indication for conducting a scoping review because of nature and pollution concerns for humanity with increased waste. Hence identification of knowledge gap for the waste management or treatment would be considered as the main purpose for performing a scoping review to develop better accessible technology. The framework opted in this scoping review was given by Arksey and O’Malley and the steps included are as follows (Arksey & O’Malley, 2005).

Identification of Research Question

As the scoping review summarizes the existing available evidence, a broad research question facilitates the inclusion of different available eggshell membrane separation methods and equipment. In this review, the broad research question was “what are the methods/techniques or equipment available to separate the eggshell membrane from the hen’s eggshell?”

Formulating the Search Strategy to Identify Relevant Studies

A systematic literature search was performed within the field of title, abstract, keywords in electronic databases of PubMed, Scopus, and ProQuest using Eggshell/Eggshells/Egg shell, Membrane, Separation/separator as keywords with AND/OR as Boolean operators. The articles published from the period of inception till 4th October 2021; published in the English language in the above-mentioned databases that provide information regarding the eggshell membrane separation technique or equipment were included. The conference proceedings, magazines, books were excluded from the study. The final search string was 1) (((EGGSHELL [Title/Abstract]) or (EGG SHELL [Title/Abstract]))) and (MEMBRANE [Title/Abstract])) and (SEPARATION [Title/Abstract])) in PubMed, 2) TITLE-ABS-KEY (EGG AND SHELL*) or TITLE-ABS-KEY (EGGSHELL*) and TITLE-ABS-KEY (MEMBRANE) and TITLE-ABS-KEY (SEPARAT*) in Scopus and 3) ti (EGGSHELL* MEMBRANE SEPARAT*) or ab (EGGSHELL* MEMBRANE SEPARAT*) in ProQuest databases.

Selection Method of Relevant Studies

The Zotero software version 5.0.96.3 was used to import and manage all the bibliographic data. After removing the duplicates, the articles were screened for the titles and the abstracts by two independent teams consisting of three reviewers each. The team examined the relevance of the articles based on the objective and the selection criteria.

Study Charting and Summarizing

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) Extension for Scoping Review flow diagram was used to showcase search results.

All included articles were charted and summarized comprehensively. The summarization included the details of the authors, Name of the Journal, Published Year, Separation Method/technique, or equipment.

Results

The search in PubMed (12), Scopus (189), and ProQuest (55) databases yielded a total of 256 articles (Figure-1). After removing the duplicates, a total of 200 articles were screened for the titles and abstracts, of which, 192 articles were excluded and eight full-text articles were screened for inclusion in the review. Out of eight, one article was excluded as it did not give any
information regarding the eggshell membrane separation technique. Finally, seven articles were reviewed. The eggshell membrane separation methods, mentioned in the literature are Microwave treatments, Acid Dissolution, Dissolved Air Floatation, grinding and mechanical stirring (Table 1 and Figure 2). Out of seven, three articles mentioned the combination of methods.

Four of the seven included articles mentioned the separation technique as manual peeling. One of the articles published in 2021 mentioned the recent patents on the separation of the membrane.

Discussion

The hen’s eggshell is made of 95% calcium carbonate by weight and the remaining part is the organic matrix (Athanasiadou et al., 2018). Waste disposal through landfill is cumbersome, space-occupying, labour-intense, and involves cost. Whereas, the burning process is labour-intensive, hazardous for the environment due to carbon emission because of the burning of protein pellicle. The calcium carbonate is non-decomposable and remains as a landfill and may leach out and lead to water and soil pollution.
Table 1. Study charting of the included studies in the scoping review

<table>
<thead>
<tr>
<th>Study No</th>
<th>Author Name</th>
<th>Journal</th>
<th>Year</th>
<th>Separation Technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A. Hussain et al</td>
<td>XVIIth World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)</td>
<td>2010</td>
<td>Manual peeling, Microwave treatment</td>
<td>(Hussain et al., 2010)</td>
</tr>
<tr>
<td>3</td>
<td>Said Nurdin et al</td>
<td>International Journal of Engineering &amp; Technology</td>
<td>2018</td>
<td>Dissolved Air Flotation Technique</td>
<td>(Nurdin et al., 2018)</td>
</tr>
<tr>
<td>4</td>
<td>Yuan Chi et al</td>
<td>International Journal of Agricultural and Biological Engineering</td>
<td>2019</td>
<td>Grinding and Mechanical Stirring</td>
<td>(Chi et al., 2019)</td>
</tr>
<tr>
<td>5</td>
<td>Garima Kulshreshtha et al</td>
<td>Biomaterials Science</td>
<td>2020</td>
<td>Manual peeling</td>
<td>(Kulshreshtha et al., 2020)</td>
</tr>
<tr>
<td>6</td>
<td>Boumediene Haddad et al</td>
<td>Chemical Data Collections</td>
<td>2021</td>
<td>Manual peeling</td>
<td>(Haddad et al., 2021)</td>
</tr>
</tbody>
</table>

Figure 2. Chart Depicting the distribution of Included articles according to the published year
The eggshell membrane contains collagen-type fibrous protein. Naturally available calcium carbonate and membrane are of medicinal value if separated, fortified, and utilized. They are also useful for industrial applications as a value-added raw material. In this regard, government agencies and research groups have taken the initiation to curb the menace of this environmental hazard in the form of separation and utilization (Projects, n.d.; Search/Department Of Science & Technology, n.d.; Waste Management-European Environment Agency, n.d.). Hence this review was performed to explore various available eggshell membrane separation methods or techniques and to delve out various commercially available equipment for utilization. Keeping the knowledge gap and non-availability of the equipment, few countries invested in research and development projects. The European Commission and the seventh Framework Program in 2012 funded a project on the separation of Eggshell membrane to attenuate the environmental problem associated with eggshell waste disposal (SHELLBRANE Separating Eggshell and Its Membrane to Turn Eggshell Waste into Valuable Source Materials., n.d.). A similar project was also executed in Canada for the repurposing of eggshell waste as a potential medical and health care product (PIC Update, n.d.).

The literature search revealed few ongoing inventions, and several technology patents regard to eggshell membrane separation. The separation of the membrane from the eggshell is a cyclic process where the proteinaceous membrane is separated from the calcinated eggshell. In this process, various methodologies at the lab scale have been developed (JP2008007419A.Pdf, n.d.; Snyder, 2016; Thoroski, 2004).

The non-chemical separation methods for separating the membrane from the eggshell include manual scraping and peeling (Haddad et al., 2021; Hussain et al., 2010; Kulshreshthta et al., 2020; Torres-Mansilla & Delgado-Mejia, 2017), mechanical methods include cyclone, venturi, and dissolved air floatation (Nurdin et al., 2018). The chemical separation is mainly through acid dissolution (Torres-Mansilla & Delgado-Mejia, 2017), microwave treatment (Hussain et al., 2010), proteolytic enzymes, etc. So that the shell and the membrane can be used individually for various biomedical and industrial applications, thereby reducing the environmental damage and empowering the hatchery and the society.

Manual Peeling or manual detachment is the simplest method of separation of membrane from the eggshell where the membrane will be slowly peeled off from the eggshell by hand without rupturing it. But this method is laborious and time taking and may not be feasible for industrial-scale utilization (“Eggshell Membrane Separation Process,” 2021).

The separation of membrane from the eggshell using microwave treatment is based on the fact that the membranes are composed of high-water content than the eggshells. They absorb more energy from the electromagnetic waves which will ultimately lead to differentiated heating of both the eggshell and the membrane. This expansion of the membranes weakens the physical connections between the eggshell and the membrane ultimately leading to separation (Hussain et al., 2010).

The method of acid dissolution is an efficient technique. The use of dilute acids like hydrochloric acid, acetic acid, sulphuric acid, and ethylenediaminetetraacetic acid dissolves the calcium carbonate of the eggshell leaving the membrane. These membranes are washed with deionized water to remove the remnants of acid. But the use of acids will be disadvantageous as some acids may alter the chemical composition of the membrane (Torres-Mansilla and Delgado-Mejia, 2017).

The method of dissolved air floatation is a recently developed technique that has a high-efficiency rate. In this method, the pressurized water is saturated with the air and is pumped into a floatation basin. The microscopic air bubbles that were formed had a role in dragging the suspended matter towards the floating surface. This entire process takes a minimum of two hours (Yoo et al., 2009).

Several inventions have been patented; the techniques like enzymatic hydrolysis (CN107043798B.Pdf, n.d.), stirring devices with pressurized water (CN103340441A.Pdf, n.d.), and friction rods (CN209268601U.Pdf, n.d.), were documented for separation. In addition to these, the use of proteolytic enzymes and the methods based on the differences in the physical properties of shell and membrane were considered for separation (Ahmed, Younes, et al., 2021; Cordeiro and Hincke, 2011).

As much as the authors are aware, this scoping review is the first of its kind that is performed on the membrane separation techniques or methods. Various commercial sites were searched for the availability of equipment but authors have not found any commercially available equipment for routine use.

Hence, the authors suggest the development of technology that fills the gap in innovation and utility. The growing agricultural (hatchery) and confectionery waste require an innovative, economic, homegrown solution for waste to wealth conversion. The authors also performed a prior art search and found few patents from various patent directories but none of them have been utilized commercially in the open market (deJong, 2013; Intellectual Property India, n.d.; New, 2021; Snyder, 2016; Vlad, 2009).

The search revealed the scarcity of literature regarding the commercially available techniques for membrane separation. At the laboratory level, few techniques were published as preliminary studies. But none of the techniques presented in the literature are freely available in the open market for routine use for translational purposes. The raw material is a value-added product if it is going to be segregated.
It will save nature, earth and water, thereby reducing environmental pollution. The biogenic raw material can be used for various biomedical and industrial purposes in an economic, viable, sustainable manner with empowerment.

Limitations of the scoping review

Discussion of techniques patented fell within the ambit of the methods mentioned priorly in the literature. The majority of the patents are design patents. The scoping review was limited to the published literature and hence the inclusion of patents for discussion in the study was avoided.

Conclusion

The key finding in this review is the availability of seven articles that mentioned the eggshell membrane separation technique or method with no specialized commercially available equipment. The methods that were mentioned in the included articles were manual peeling, microwave treatment, acid dissolution, dissolved air floatation, and the use of proteolytic enzymes. The technology gap needs to be filled for the betterment of mankind and nature through rigorous research and development from waste to wealth, which is the need of the hour.

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