STUDY ON UTILIZATION OF AGRO-WASTE AS SOURCE FOR HEPARIN

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Dedicated

To

My Beloved Parents,

and Supervisor

CONTENTS

ABSTRACT

Heparin is a highly sulphated and most negatively charged natural biopolymer belonging to the glycosaminoglycan (GAG) family. This is about a 100-year-old anticoagulant drug. This is equally important for non-anticoagulant diseases also and is the reason for the recent burgeoning of interest in the molecule. Heparin has been isolated from both animal and non-animal sources; however, porcine mucosa remains the FDA-approved source for heparin. For the synthesis, chemical, chemoenzymatic, and biotechnological approaches have been studied. In recent times, the focus is more on synthesizing LMWH, ULMWH, and bioengineered heparins.

A sustained and healthy society needs proper utilization of the waste material to deal with the increasing pollution rate. Several works are successfully done on this note, and till now, several strategies have been developed for the production of bio-chemicals from biological waste. In other words, value has been added to the waste materials. The biochemicals like starch, maltose, amylose, etc. have been isolated from the biological waste. These biomolecules may be used as a source of heparin synthesis to avoid the synthetic dependency of living organisms.

Here in this work, I have explored the possibilities of biomolecules isolated from waste as source of heparin synthesis.

List of Figures

1. INTRODUCTION

Heparin (Figure 1) is a natural biopolymer belonging to the glycosaminoglycan (GAG) family [1-3]. This is a highly sulphated and most negatively charged polysaccharide. The heparin polysaccharide has disaccharide repeating units, where the disaccharide is 1,4-glycosidically linked and consists of uronic acid residues and D-glucosamine (GlcN). The uronic acid residue is either β-D-glucuronic acid or α-L-iduronic acid (IdoA) [4]. The GlcN is further substituted with an N-acetyl (GlcNAc) or an N-sulpho (GlcNS) group, which can also be O-sulphated at the 6-position [1]. This molecule is heterogeneous in nature due to structural variations, which help to interact with different proteins leading to various biological activities. Some of the important properties of heparin are summarized in Figure 2 [5,6]. Some of the important brands of heparin available with their generic name asenoxaparin, dalteparin, tinzaparin, heparin flush, danaparoid.

Many researchers have described the discovery and development of heparin. The three persons, William Henry Howell, Luther Emmett Holt (Jr), Jay McLean, names were involved in the discovery of this molecule in 1916-1918 [7,8]. After about 14-15 years of work, this biopolymer was considered as an effective anticoagulant drug [9]. Further, medicinal investigation proved that heparin is equally potential as a pharmacological molecule for various non-anticoagulant diseases also [6,10-12].

Being a biopolymer, heparin is biosynthesized by the concerted action of about 22 enzymes which reflects its complexity towards synthesis. This is biosynthesized in the endoplasmic reticulum (ER) and Golgi [13-16]. This molecule is isolated from porcine intestinal mucosa or bovine sources [17,18]. The isolation of this pharmaceutic is very tedious, with a low yield of about 180-260 mg per healthy animal. One of the major limitations of its production is that this molecule is mainly obtained from a single animal species. A lot of research has been done towards finding the other animal and non-animal

sources with very little or no success. In non-animal source finding research, chemical and chemoenzymatic synthesis have been tried with very limited success. A most successful example of chemical synthesis is the chemical synthesis of Arixtra. The synthesis of this molecule takes place in about 50 steps with an overall yield of ~0.1% [19,20]. However, this is also not purely chemical synthesis.

"According to government data, the annual market for heparin is about Rs 50 crore for almost 63 lakh units and Rs 504 crores for enoxaparin, which is for about 150 lakh units [4]. These estimates are of the product imported into India, which is about 2,500-3,000 kilos for Heparin from which about 1,000 Kgs of enoxaparin is processed in India. This doesn't account for the product that is imported directly as the finished product [21-23]."

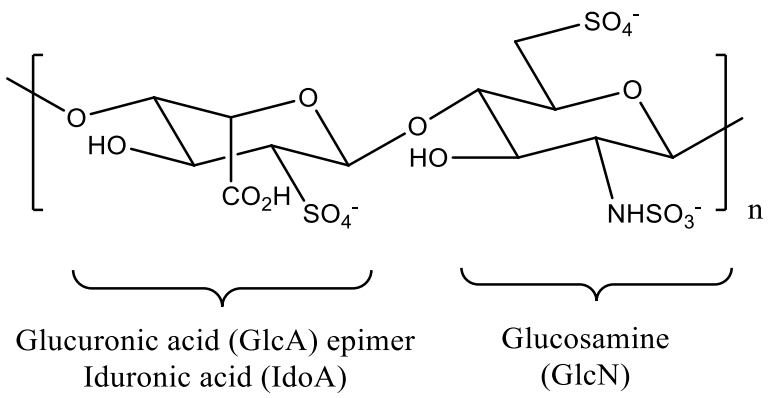

Figure 1. Maximum disaccharides in heparin

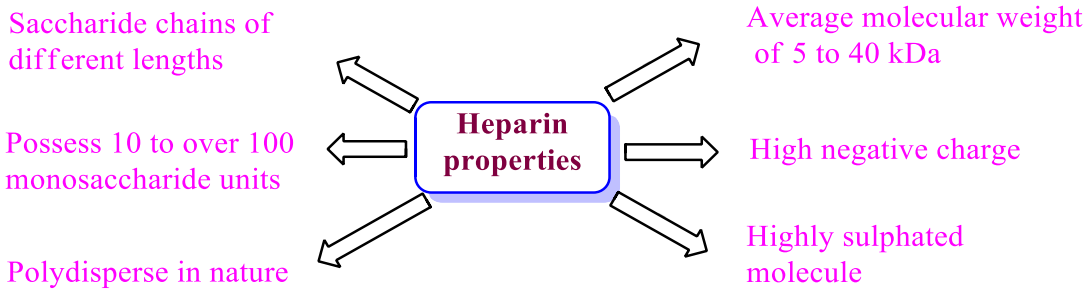

Figure 2. Important properties of heparin

2. ISOLATION AND SYNTHESIS OF HEPARIN

2.1 Isolation of heparin

Any animal used as the source for any drug isolation has to follow strict ethical guidelines. For heparin isolation also, the health and medication of slaughtered animals have to meet certain requirements [24]. There is full traceability from farm to pharma is maintained with proper regulations [24-26]. The earlier heparin isolation was done with canine or bovine livers; later, bovine lungs and mucosa were used (Figure 3) [18]. However, due to the outbreak of mad cow disease (Bovine Spongiform Encephalopathy (BSE)) [27], the use of bovine materials for heparin isolation decreased significantly [28]. The porcine mucosa remains the FDA-approved source for heparin; however, FDA encourages reintroduction of bovine-source also [29]. There is a strong requirement for enhanced knowledge about bovine disease and the heparin purification process to reduce the risk associated. Studies have shown that the bovine heparin is less active than the porcine heparin; this may be due to their structural differences between the two [30].

The sheep (ovine) intestines have also been studied as a source to isolate pharmaceutical heparin [28]. Ovine heparin showed better disaccharide composition than the bovine heparin [31,32]. Further, the use of ovine is also free from any religious sentiments. However, the disease like scrapie in sheep found to have some concern, although this is not transmissible to humans.

Many mammalian sources have been studied to isolate heparin [33,34]. The human tissues were used as a natural source of heparin [35,36]. The frozen hemangioma tissue gave human heparin in the amount 649 µg/g of tissue [35]. Warda et al. isolated heparin from one-humped camel in 400 mg/kg amount from an adult camel [37]. This method and source gave better results in comparison to the source porcine intestine (250 mg/kg).

Among the non-mammalian sources, poultry, molluscs, fishes etc. have been explored to isolate heparin. Chicken intestines produced heparin with a lower degree of sulphation [30]. Heparin was also isolated from the turkey intestine but showed very poor activity [38]. Till today, to the best of my knowledge, there is no industry using poultry by-products to isolate heparin. In another study, tuna (saltwater fish) skins have been utilized to isolate heparin with the help of anion exchange resin [39]. Through steaming, the skin was obtained from the freshly caught tuna fish. The obtained heparin showed less potency than the commercial product [39]. Heparin was also isolated and purified from gills and intestines of salmon (*Salmo salar*) whose activity showed similarity with the activity of low-molecular-weight heparin (LMWH) [40]. The heads of shrimp (*Penaeus brasiliensis*) produced LMWH with a yield of32 mg/kg [41]. The isolation from clams (*Tapes phylippinarum*) gave heparin with a yield of ~2.1 g/kg dry tissue [42]. The commercial isolation/production from the discussed sources (Figure 3) is still a matter of research.

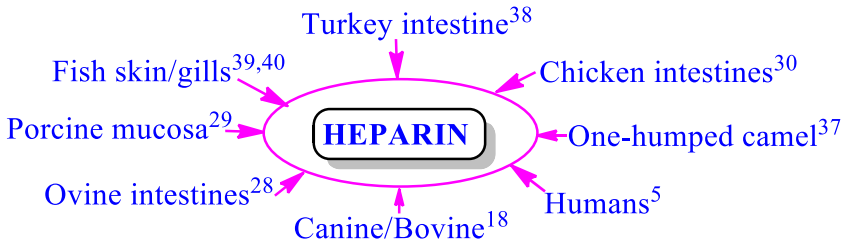

Figure 3. Natural sources of heparin

2.2 Synthesis of heparin

The synthesis of heparin is very challenging, and still a lot of work is going on towards this. Heparin of various polysaccharide chains has been synthesized/semi-synthesized [43-46]. As per the length and molecular weight, heparin is grouped as unfractionated heparin (UFH), low molecular weight heparin (LMWH), and ultra-low

molecular weight heparin (ULMWH). Chemical synthesis and chemoenzymatic synthesis have been tried with limited success. In both the methods, oligosaccharide building method has been explored. The methods are multi-steps, taking place in more than 50-60 steps leading to very-very low yields. For example, a heparin, fondaparinux, was synthesized in about 60 steps with an overall yield of only 0.1% [47,48]. Fondaparinux is a pentasaccharide sequence having a similar binding region as heparin. The challenge associated with heparin synthesis are many like repetitive protection deprotection steps, couplings, functional group activation, intensive purification, and so on [1,49].

The chemoenzymatic synthesis takes the help of polymerases which help in backbone building. The backbones have further been modified with enzymes like C5 epimerase and sulfotransferases [50]. The backbone of the chemoenzymatic method is the process involved in its biosynthesis; rather, one can say it is mimicking the biosynthesis of heparin [51-53]. Xu et al. synthesized two ULMW heparins through a chemoenzymatic approach [53]. The focus of the synthesis is to make the pharmacophores of anticoagulant heparin [54].

Another chemoenzymatic synthesis used the bacteria *E. coli* K5 [55]. This gave an unsulphated precursor of the heparin, heparosan. The chemoenzymatic method came with a lot of limitations, like substrate specificities of the enzyme.The heparin-based oligosaccharide has also been synthesized using the polymer-supported method [45,56]. However, the glycosylation yield enhancement remained a challenge, and hence solution phase is still favoured.

The chemical synthesis of heparin is not like the total synthesis of heparin. Most of the researchers have synthesized the active portion of the chain like pentasaccharides or hexasaccharidesas heparin-based oligosaccharide and further evaluated for the corresponding biological activity. This is an excellent approach to get the potential pharmaceutical active molecule with comparatively lesser effort.

3. MEDICINAL APPLICATIONS OF HEPARIN

Heparin for the first time used as postoperative anticoagulation in 1935, and since then, their applications have increased many folds [57]. The utility of heparin, directly or indirectly, in other therapy like vein thrombosis, heart-lung oxygenation, kidney dialysis, and coating of medical devices like stents make this molecule vital for medicinal applications.

3.1 Anticoagulation effect of heparin

A blood clot known as a thrombus is consists of fibrin and blood cells, which can form anywhere in the cardiovascular system [58,59]. The clot can be in the microcirculation, the heart, arteries, and veins. Many diseases are associated with thrombosis including, cardiac-related disease [60].

Heparin is used for the treatment or prevention of thrombosis, re-thrombosis after thrombolysis, and other thrombosis-related diseases [10]. The blood-clotting cascade is inhibited by a serine proteinase inhibitor, anti-thrombin III (AT) [61]. AT is a plasma protein and named by Abildgaard in 1968 as anti-thrombin III, which later referred to as anti-thrombin (AT) [62] after the study of Brinkhous et al. that heparin requires a plasma cofactor for its activity [63]. A pentasaccharide sequence (Figure 4) present in heparin is responsible for the anticoagulation effect [64,65]. The sulfo- and carboxyl groups possessing the negative charge bind tightly with AT [66,67]. AT itself is a weak protease inhibitor, but in combination with heparin pentasaccharide, increase its activity by about 300 times due to favourable conformational change [68]. The formation of insoluble fibrin clots from soluble fibrinogen is facilitated by thrombin. This step is blocked due to the formation of a complex AT-heparin-thrombin, and hence the formation of an insoluble fibrin clot is avoided [69,70].

As per the mechanism of action, a number of coagulation factors such as XIIa, XIa, IXa, Xa, and IIa (thrombin) are inactivated by the heparin-AT complex [6,71-73]. The

unfractionated heparin (UFH) possesses low bioavailability (30%) in comparison to LMWHs (90%) and a longer half-life (17–21 h) when compared to UFH. The LMWHs are given for thromboprophylaxis in fixed doses [73,74]. Due to low bioavailability, the doses need to be high, like 30,000 U/day, when administered via subcutaneous injection for therapeutic anticoagulation [73].

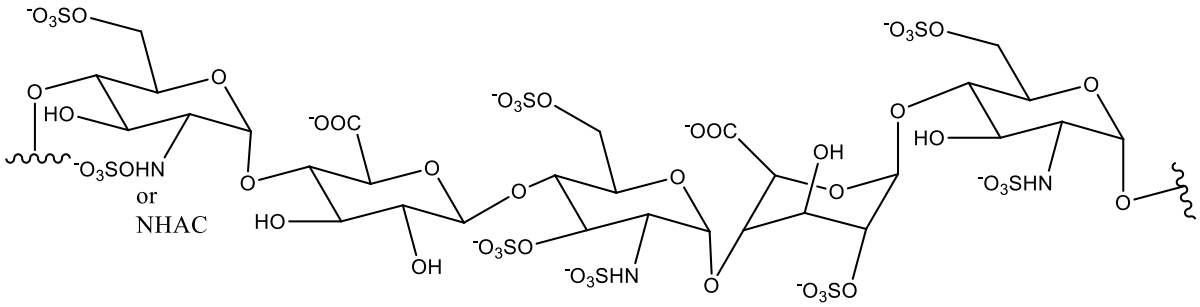

Figure 4. Heparin pentasaccharide that bind to AT

In an ongoing pandemic, COVID-19 also, heparin has been widely explored for its utility, which includes both its anticoagulant effect and antiviral effect [10]. The study showed that anticoagulant therapy with heparin has a positive effect and lowered mortality of COVID-19 patients. The COVID-19 patients with higher D-dimer levels or met the criteria of sepsis-induced coagulopathy (SIC) better impacted with heparin therapy [75]. Patients with 4-times higher D-dimer concentrations than normal were recommended for LMWH [76]. Some studies also confirmed the effectiveness of heparin in COVID patients with venous thromboembolism prophylaxis [77,78].

3.2 Anticancer effect of heparin

The detailed studies of unfractionated and different molecular weight heparin showed improved effects on various types of cancer [11,79-81]. The cancerous tumors and thrombosis showed some link between them; this led to the use of antithrombotic molecules

towards the treatment of cancer. The mechanism of action is still a matter of investigation; a noncoagulation pathway is a possible suggestion [82]. The study with heparin on animal models showed a reduction in metastasis of carcinoma cells [83-85]. This may be due to the non-deposition of fibrin around tumur cells [83]. The inhibition of biological processes that supports cell growth is an important approach to target cancer [86]. In some studies, heparin possessing non-anticoagulant property also inhibited metastasis [84,85].

Several studies confirmed that heparin has effects on angiogenesis and also affects the formation and progression steps of the tumor [87]. They regulate the immune system due to their ability to bind with different proteins. Their role in cell adhesion, a barrier to leukocyte migration, and inflammation have been studied [11,88]. Based on various studies, several heparin-like molecules or heparin mimetics such as pixatimod, muparfostat, necuparanib, and roneparstat are currently in clinical trial for use in cancer treatment [11]. Heparin was used to study its impact on the treatment of angiogenic tumours in mice [89]. The result showed inhibition in downstream signalling and decreased angiogenesis. The heparin, by competition, interrupts the heparin sulphate proteoglycans interaction with fibroblast growth factors (FGF) [89].

The heparin sulphate is present in the extracellular matrix plays several characters towards cell division and proliferation, such as a positive or negative modulator [90]. This has been observed that proliferation of many cells is inhibited by heparin, and this behaviour is utilized for the treatment of cancer [91]. Due to the positive results obtained after many studies for cancer treatment by heparin or its derivatives, more than 50 clinical trials are at different stages. However, the overall beneficial nature of these drugs is still a matter of investigation.

3.3 Antiviral effect of heparin

The GAGs are present on the cell surfaces, which allow them to serve as a non-specific receptor for virus binding [92]. Various studies have been done with heparin against a crowd of distinct viruses [10,93-95]. The human immunodeficiency virus (HIV) showed a reduction with heparin towards cytopathogenicity to MT-4 cells by preventing the adhesion of HIV to MT-4 cells [96]. The heparin-sulphate made the dengue virus ineffective by inhibiting the virus-cell attachment [97]. This has been observed that the heparin blocks the virus adhesion by competitive inhibition [98]. A study by Lin et al. showed the inhibitory action of heparin towards replication of Japanese encephalitis viruses in BHK-21 cells and dengue-2 in hepatoma [99]. The study was done with heparin at various dosages like 0.1, 1, 10, and 100 mg/ml). The result showed that the dengue-2 virus invasion can be stopped by heparin in different liver cells. The influenza virus strain H5N1 infection was also prevented by heparin and its derivatives [100]. The N-sulphation of HS showed its potential towards the infectivity of the Chikungunya virus [101].

The heparin also showed its effectiveness towards SARS-CoV-2 [10]. The angiotensin-converting enzyme 2 (ACE-2) is the major receptor, whereas heparan sulphate proteoglycan (HSPG) is the co-receptor for the virus, SARS-CoV-2, which this virus utilizes to infect the cells. Heparin inhibits the binding of SARS-CoV-2 to the cell surface by competing with co-receptor HSPG, and hence, stops the virus entry to the cell [10,102]. Conzelmann et al. studied SARS-CoV-2 infection inhibition using heparin as a repurposed drug [103]. The study was based on three important therapeutic actions of heparin, anti-inflammatory, anticoagulant, and antiviral. The results showed that the viral replication was completely inhibited with 500–1,000 μg/ml heparin and get suppressed to 60% at 125–250 μg/ml [103].

3.4 Antibacterial activity of heparin

For the first time, Stoker reported the antibacterial effect of heparin against *Staphylococcus aureus* [104]. One year later, another group also reported the antibacterial effect of heparin against *S. aureus* and *Erwinia stewartia*. [105]. Hanno et al. in 1978 studied the effect of heparin as an antibacterial agent on rabbit bladder [106]. The mucoprotein-deficient bladder was applied with heparin directly gave a very good result towards bacterial infection. Rosett and Hodges studied the effect of heparin on eight species of microorganisms in brain heart infusion broth [107]. It was observed in all the studies that the microorganism growth was inhibited by heparin. The gram-positive bacteria got inhibited more than the gram-negative bacteria. The unfractionated heparin showed inhibition in a dose-dependent manner to three of seven *S. pneumoniae* isolates and one of five *H. influenzae* isolates [108]. The heparin coating also showed antibacterial properties. The heparin-coated stents prevented the formation of bacterial biofilm and microbe attachment [109]. The biodegradable ureteral stents coated with heparin were also found to inhibit the early bacteriuria development [110]. The above examples confirm the utility of heparin as antibacterial agents. Systematic research is required to bring this as a broad-spectrum antibacterial agent.

3.5 Anti-inflammatory activity of heparin

Several studies have been done on heparin as an anti-inflammatory agent [111-113]. Different studies have discussed the mechanism of action; still, exact benefits and safety are yet to be established about heparin being an anti-inflammatory agent. Some of the early studies have suggested the positive use of intravenous heparin in asthma, a chronic inflammatory disorder of the airways [114,115]. The inhalation of heparin also gave positive effect towards bronchial hyperreactivity [116]. The heparin inhalation may be showing the suppressive type of activity on mast cell degranulation which is due to the absence of

bronchodilation in airways [116]. In the burn's patients also, heparin was able to reduce the inflammation and also promoted tissue repair [117].

Heparin and its derivatives showed anti-inflammatory property and benefits to the patients during cataract surgery, cardiopulmonary bypass, and asthma; however, other inflammatory diseases gave mixed and inconsistent results [113]. The adverse effect of heparin as anti-inflammatory agent has not been reported in maximum studies. Still, more studies are required to make this molecule as a versatile and robust anti-inflammatory agent.

3.6 Heparin-based nanocarriers for Drug Delivery

Heparin is biocompatible, and hence, heparin-based nanocarriers showed a lot of potential in drug delivery [118,119]. Different types of nanocarriers have been studied with heparin like self-assemblies, coated nanoparticles, nanogels, and polyelectrolyte complex nanoparticles [119]. The size and shape of heparin-based nanoparticles can easily be controlled by optimizing the heparin amount. The physical or chemical interaction using the cross-linked method allows the formation of heparin-based nanogels possessing 3-dimentional (3-D) porous network, which helps in the prevention of environmental degradation of drugs along with drug reservoirs. Chang et al. developed a heparin/berberine conjugate to deliver berberine at the infected site that reduced cytotoxic effects in infected cells [120]. This process reduced the unwanted side-effects caused due to antibiotic treatment for *Helicobacter pylori* bacteria. This was an excellent example of a nanoparticle berberine carrier with a heparin shell [120]. Choi et al. studied the controlled release of growth factors using heparin nano-sponge [121]. The growth factors are useful to many diseases therapy and tissue engineering, but due to low bioavailability, non-specific biodistribution, and fast degradation, their efficacy is a limitation. These limitations are reduced with the use of heparin-based nanocarriers.

Heparin-based nanocarriers have been studied for cancer management either as a backbone, coating material, physical encapsulation, and conjugates [119]. An elevated antitumor effect was observed with doxorubicin encapsulation by a conjugate made from heparin and deoxycholic acid that was planned for SCC (squamous cell carcinoma) [122]. This was amphiphilic conjugate nanoparticles and gave a better result than without encapsulation. Zhang et al. studied conjugation and physical loading for two anticancer drugs, D (doxorubicin) and A (all trans retinoic acid) where D was loaded, and A was conjugated with LMWH forming D-loaded LMWH–A system [123]. The study reported that the anticancer effect with the prepared system was higher as compared to the free drugs in solution. Yang et al. studied the applicability of drug sorafenib in gastric cancers using an immobilized chitosan/heparin pluronic-coated system as a nano delivery system [124]. This was observed that the prepared nano delivery system enhanced the inhibition of cancer cells.

There have been numerous studies done to use heparin as a nano system for drug delivery, but still, no system has reached to the stage of successful clinical trials. Heparin being the hydrophilic molecule possessing a lot of modifiable groups, can provide an excellent potential in the drug delivery systems.

4. INTRODUCTION TO AGRI-WASTE

Wastes are anything that is not of humans use. Waste management (WM) is a serious problem for both urban and local bodies. Effective WM is the requirement of sustainable life. There are various effective methods that can be applied for better WM (Figure 5) [125]. There are a lot of barriers to effective WM, which posed both challenges and opportunities for the researchers to work scientifically in this area. The waste has been classified and analyzed on the basis of different aspects; the important one is the basis of the source [126].

Biological waste is usually grouped as wastes that is generated from natural sources. One of the most talked, and researched natural waste is agricultural wastes. The important types of agricultural wastes include field-based waste, associated animal waste, and agro-industrial wastes [127]. Agricultural products like crops, fruits, vegetables many times become agricultural waste either as raw material or during the processing of raw materials. Food waste is an example of agro-industrial waste. They are produced through agricultural operations or household consumption.

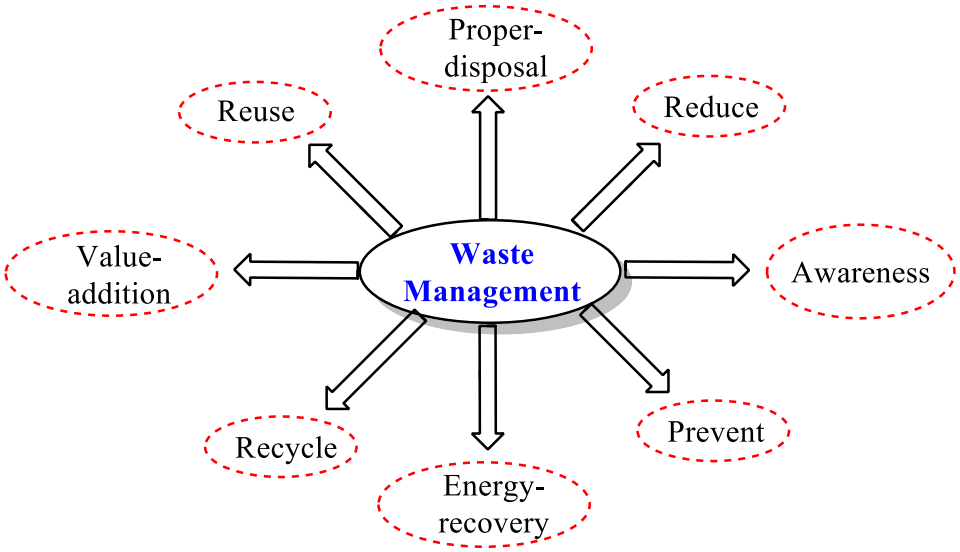

Figure 5. Methods of waste management

5. GENERAL INFORMATION ABOUT FOOD WASTE

Sustainability is much needed for surviving. Sustainability means configuring and planning the present-day human activity so that the future generation will not face any difficulty. Due to the increase in the global population; there is a proportional increase in food requirements [128]. The increase in consumption of material is proportional to the increase in wastage. It was estimated in 2011 by the Food and Agriculture Organization of United Nation (FAO) that the food waste per year is about 1/3rd of the world's food [129].

It was reported that the discarded food waste is more than enough to feed 815 million hunger people globally. In general, food wastage can be any type of food loss or food waste. Although both the terms are used, they seem to be identical but quite different, employing definition and meaning. According to FAO "Food loss is the decrease in the quantity or quality of food resulting from decisions and actions by food suppliers in the chain, excluding retailers, food service providers and consumers."

In simple word, food loss is defined as any food that is disposed or discarded unenviably along the supply chain of food at the time of harvesting or storage or transportation. This does not include the retail level, and any other productive utilization. "Food waste refers to the decrease in the quantity or quality of food resulting from decisions and actions by retailers, food service providers and consumers." However, it can be wasted through many ways:

- Fresh product that are discarded during sorting operations in terms of color, shape or size or any type of consideration that is not fulfilled by the product.
- Expired or close to expiry date food products are mostly discarded by retailers and consumers.
- Large quantities of unused or left-over edible foods are discarded from household kitchens or from eating establishments.

As production of food is resourceful so when food is discarded, it is not just the food that has been discarded, but all the water, land, hard work of the farmer or laborers, fuels, transport costs, everything is discarded. Moreover, food wastage also causes lots of environmental impacts like soil erosion, deforestation, water and air pollution. Also, after disposal or at the time of production, also it causes the emission of greenhouse gases. It is calculated that the amount of food that is discarded is produced in approximately 28% of the world's agricultural area i.e., 1.4 billion hectors of agro-based land (annually) [125].

In recent decades, food waste has become an emerging area of interest from urban, rural, national and international level organizations and policymakers. Many research centers, academics, NGOs show their concern about utilizing food waste as valuable resources of many bio-active molecules and for minimizing its harmful environmental effects like soil erosion, deforestations, water, and air pollution & most importantly the greenhouse gas emission [130]. Food waste has become global problem now-days. Many factors like slow progress in the development of effective waste management, public carelessness, easy excess to food, much wealthier to afford sufficient food and unnecessarily throwing them into garbage, while still being consumable [131].

However, food waste is generated all along the food supply chain, but at the household level highest amount of food waste is generated [132]. Million tons of food waste is generated globally, and it is very hard to quantify each of them and control them in a recycling manner in a due short time. However, we can manage to utilize them as resources of some of the important bio-active molecules like complex carbohydrates, proteins, lipids, and nutraceuticals [133]. Some studies reported that the extraction of bulk chemicals from food waste is more profitable than converting into biofuels [134]. Based on the above facts, in this paper, the extraction of bio-active molecule, carbohydrates mainly starch and maltose, from food waste, a biological waste material, have been compiled. This study will help in food waste management and isolation of useful chemicals from them.

6. SOURCES OF FOOD LOSS

From farm to home, in every sector i.e., during harvesting, processing, transportation and distribution, in restaurants and even at home food is wasted along the food chain.

6.1 Food loss on farms

Farms are the first place of food chain. However, many times farmers harvest more crops on seeing the demand of consumer but due to so many reasons like lack of storage facility, lack of transportation facilities or sometime due to sudden down of market demand of that product leads to wastage. Another unavoidable source of food waste from farm is the unwanted parts of the crops like in case of wheat only the seed is consumed but other parts like head, stem, leaves and roots are generated as waste material. It was found that 20 billion pounds of product is lost on farms every year [131]. In recent cases due to the COVID-19 pandemic lockdown has occurred which causes a huge loss to the farmers along with creating lots of food waste.

6.2 Food loss during Manufacturing

Food waste at manufacturing sector mostly causes at the time of trimming off the edible portions like peels, skins, fats or crusts from food. Although some are used to feed the animals and some are reused but most of the parts are just thrown away which ultimately causes serious environmental issues. For example, in the juice factory the peel of orange and mango are discarded. Sometimes overproduction, product damage and some kind of technical issues, production trials, packaging defects, trial runs, and wrong sizes and weights lead to food waste in a large quantity [131,135].

6.3 Food loss in Transportation & distribution

Food waste during transportation is most vulnerable mostly in Developing countries as there are lack of good transportation means, adequate and reliable storage system and good infrastructure. However sometimes due to carelessness of the driver accidents are occurred and cause both food loss along with life loss.

6.4 Food waste in retail business

Retail stores or supermarkets are another source of food waste. The main drivers for food loss in supermarkets are overstocked products, colour, shape and size issue, damaged product, expired products etc.

6.5 Food waste in restaurants and Home kitchens

Restaurants and home kitchens are the main source of food wastage. Households are responsible for the largest portion of all food waste.

7. VALUE-ADDITION TO FOOD WASTE

Food waste is present everywhere and many valuable molecules have been isolated from this [136-139]. Traditionally, they have been disposed only through landfills but with the development of analytical techniques and isolation methods, values have been added to them. This is an important part of food waste management. The biochemicals like starch, maltose, amylose etc. have been isolated from the biological waste. Starch is anion-reducing homo-polysaccharide consisting of 10 D-glucose units; can be amylase and amylopectin whereas, maltose (4-α-D-glucopyranosido-D-glucopyranose) a reducing sweet white crystal which is a disaccharide; consisting of 2 D-glucose unit linked by glycosidic bonds. Starch upon hydrolysis by enzyme like amylase forms maltose.

Torres et al. used low sized or irregular shaped discarded potatoes from three varieties like agria, kennebec, and neiker, as raw material for extraction of starch through subcritical method. The % of starch yields are 24.4 for agria, 22.1 for kennebec and 18.5 for neiker [140]. Li et al. extracted starch from core and pericap of fallen ripen kiwifruit with yield % 34.6 to 40.7 and 38.6 to 51.8 respectively [141]. Shehzad et al. extracted maltose by utilizing food waste like damaged wheat grain with 21.7 % yield [142]. Nakthong et al. used

waste pineapple stem as a significant resource for the extraction of good amount of starch by mechanical extraction method. The % yield of starch and amylose are 30.0 and 34.4 respectively [143].

Guo et al. isolated starch from the kernels of jack fruit, longan, loquat, and litchi and mango fruits. The % of yield of starch from litchi is 53 and from longan and loquat yield % are 59.0 & 71.0 respectively [144]. Jaiswal et al. isolated starch from shahi litchi seeds using both acidic and alkaline extraction method got 11% and 12.6% yield respectively. (145) Lists of different food waste and carbohydrates isolated from them are listed in table 1.

Table 1. Carbohydrates isolated from food waste

S. No.	Source	Waste residue	Carbohydrates (%)	Reference
1	Potato	Low-sized or irregular shape discarded potatoes from Agria	Amylose (29.3)	[140]
2	Potato	Low-sized or irregular shape discarded potatoes from Kennebec	Amylose (27.3)	[140]
3	Potato	Low-sized or irregular shape discarded	Amylose (23.0)	[140]

		potatoes from Neiker		
4	Fallen kiwifruit during ripening	Core tissue	Starch (38.6-51.8)	[141]
5	Fallen kiwifruit during ripening	Pericarp	Starch (34.6-40.7)	[141]
6	Fallen kiwifruit during ripening	Core tissue	Amylose (15.5-17.8)	[141]
7	Fallen kiwifruit during ripening	Pericarp	Amylose (20.7- 23.3)	[141]
8	Mango	Kernel flour	Starch (44.9)	[143]
9	Mango	Kernel flour	Amylose (9.1-16.3)	[143]
10	Pineapple	Stem	Starch (30.0)	[146]
11	Pineapple	Stem	Amylose (34.4)	[146]
12	Mango	Kernel flour	Starch (44.9)	[147]
13	Mango	Kernel flour	Amylose (9.1-16.3)	[147]
14	Mango	Kernel	Starch (75.6-80)	[148]
15	Litchi	Seeds	Starch (53)	[145]
16	Litchi	Seeds (acidic method)	Starch (11) Amylose (9.60)	[144]

		Seeds (alkaline method	Starch (12.6) Amylose (7.60)	
17	Litchi	Seeds	Amylose (19.2)	[149]
18	Jackfruit	Seeds	Starch (60-80) Amylose (22.10-38.34)	[150]
19	Tamarind	Seeds	Amylose (14.2)	[151]
20	Longan	Kernel	Starch (59.0)	[144]
21	Loquat	Kernel	Starch (71.0)	[144]
22	Annatto	Seeds	Starch (66) Amylose (24)	[135]
23	Avocado	Seeds	Starch (27.5-29.8) Starch (wet milled) (19.7)	[152]
24	Apple	Immature apples	Starch (44-53) Amylose (26-29.3)	[153]
25	Banana (unripe)	Pulp	Starch (70-80)	[154]
26	Banana (unripe)	Peel	Starch (29)	[155]
27	Banana (unripe)	Flesh	Starch (69.5) Amylose (21.3)	[156]
28	Banana (unripe)	Peel	Starch (22.6) Amylose (25.7)	[157]
29	Apple	Dry apple	Pectin	[158]

			(10-15)	
30	Citrus fruits		Pectin (20-30)	[158]
31	Apple	Pomace	Carbohydrates (48-62)	[159]
32	Orange	Peel, pulp, seeds	Carbohydrates (47)	[160]
33	Pear	Pulp	Carbohydrates (62.8)	[161]
34	Cabbage	Frozen edamame	Maltose (9-12)	[142]
35	Wheat	White wheat flour	Maltose (2.2- 9)	[142]
36	Barley	Spaghetti	Maltose (19.6)	[142]
37	Wheat	Damaged wheat grains	Maltose (21.7)	[142]

8. SUMMARY AND WAY FORWARD

Heparin is a highly sulphated and most negatively charged natural biopolymer belonging to the glycosaminoglycan (GAG) family. This is about a 100-year-old anticoagulant drug, and still, the scientific community unable to find its substitute with similar efficacy. Various bottlenecks towards its isolation from animal sources kept the scientific community at their toes to search for similar alternatives. From publications point of view, many researchers have published its isolation from different sources, chemoenzymatic synthesis, chemical synthesis, biotechnological approach, but still, their commercial applicability is far away.In recent times, the focus is more towards the synthesis of LMWH, ULMWH, and bioengineered heparins.

Heparin is equally important for non-anticoagulant diseases and nanocarriers for drug delivery systems also. The effect of heparin and its oligosaccharides showed a better therapeutic effect towards cell proliferation, inflammation, microbial effect, thrombogenesis,

and related diseases. More in-vitro, in-vivo, and clinical studies are required to understand the efficacy and treatment effect of heparin-based oligosaccharides, which are easy to synthesize in comparison to the total synthesis of heparin.

To develop the eco-sustainability, value addition to waste materials is important and widely used in recent times. However, there is still lot of research is required to isolate biochemicals from waste. The use of food waste for the isolation of biochemicals has been exploited by many researchers, which reduces the burden on landfill. The commercial exploitation is not very prevalent due to the cost of production. Keeping this in mind, this field is wide open to work.

The heparin may be synthesized using the proposed protocol (Figure 6):

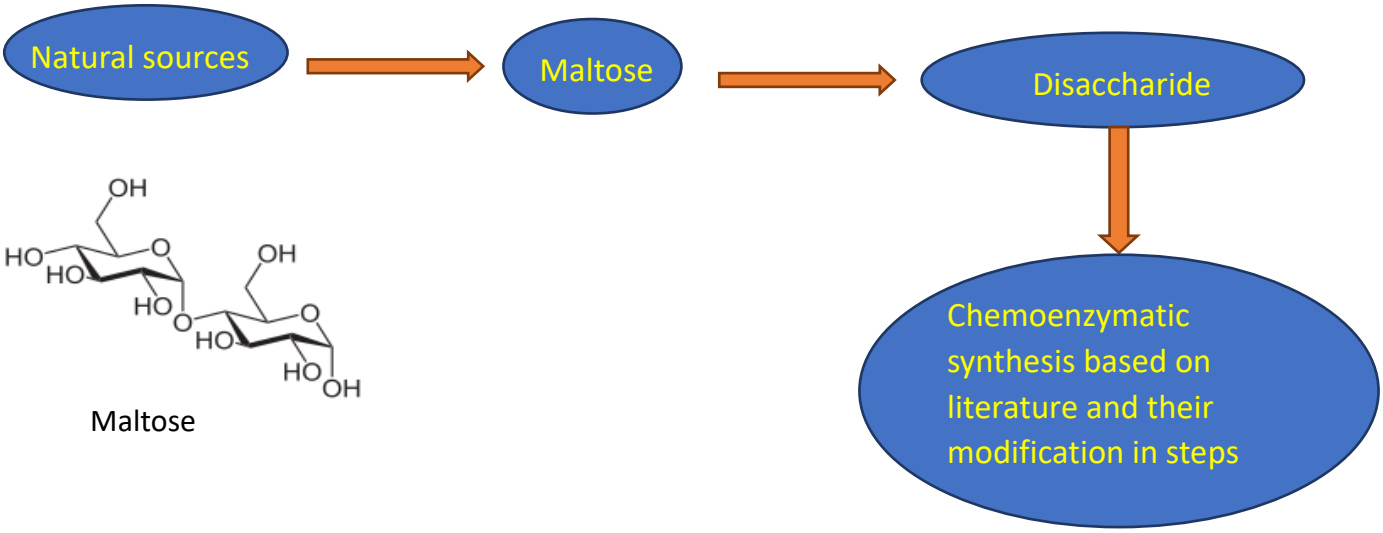

Figure 6. Protocol for heparin synthesis

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10. PUBLICATION

Peer-reviewed Journal:

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Saikrushna Jena and Ram Singh; Carbohydrates (starch and maltose) from biological waste Materials; 710-722, 2021, ISBN: 978-93-5457-142-8; International conference on Green Technology for Sustainable Development (GTSD2021), organized by Dharmsinh Desai University, Nadiad, India from 9-11 March **2021**.

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