



**DEVELOPMENT OF NATURAL BROWSING REPELLENT TO PROTECT
GARDEN AND FOREST PLANTINGS**

Lappeenranta–Lahti University of Technology LUT

Master's thesis

Erasmus Mundus Joint Master Programme in Sustainable Biomass and Bioproducts
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Examiner(s): Associate Professor, Kristian Melin

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ABSTRACT

Lappeenranta–Lahti University of Technology LUT

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SBBE master's degree Programme, in co-operation with partner university/universities: Wrocław University of Science and Technology, Poland and University of Castilla–La Mancha, Spain.

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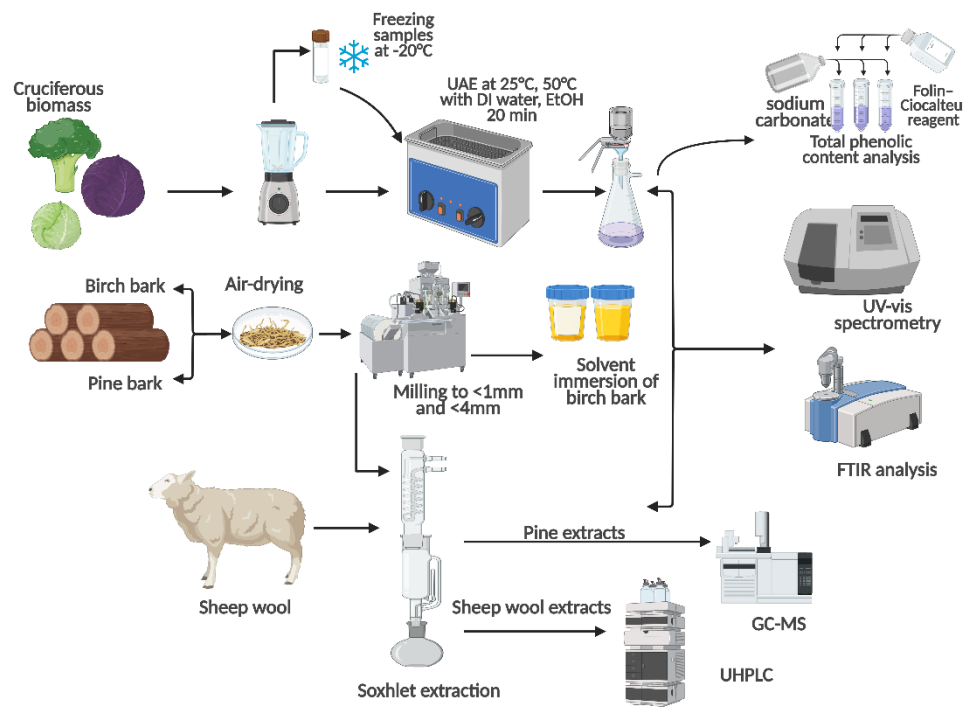
Browsing by moose and roe deer causes significant damage to Finnish forests and garden vegetation. The current synthetic repellents present environmental and economic drawbacks, while existing natural repellents show limitations in persistence and effectiveness. Hence, developing natural alternatives from locally available biomass offers a sustainable approach to reduce animal browsing while valorising agricultural and forestry side streams.

This study aims to characterise cruciferous vegetables, birch and pine bark, and sheep wool grease as potential raw materials for natural repellent formulations. Extraction of bioactive compounds was carried out by ultrasound-assisted extraction for cruciferous biomass, and by Soxhlet extraction for wood barks and sheep wool grease. The obtained extracts were characterised with UV-vis spectroscopy, FTIR, GC-MS, and HPTLC. Total phenolic contents of cruciferous biomass extracts were also measured.

Results indicate that broccoli showed the most promising deterrent potential among crucifers with high potentially sulforaphane- and glucosinolate-related absorbance (196 and 227 nm) and optimised release of sulphur volatiles after freeze pretreatment (at -20 °C) with UAE at 50 °C. Birch bark extracts contained triterpenoids such as betulin (with UV absorbance at 200–230 nm, and infrared peak at 887 cm⁻¹). Triterpenoids of birch bark are characterised by bitterness and persistence properties, whereas pine bark extraction resulted in higher contents of lipophilics (64 mg/g) consisting of resin acids, fatty acids, and esterified lipids which are associated with odour deterrence. The extracted sheep wool grease contained 55–60 wt% free fatty acids/alcohols, 10–20 wt% sterols, and 10–15 wt% wax esters, which makes it more persistent due to its film-forming hydrophobicity compared to commercial Trico repellent, which contained 99 wt% triglycerides.

These findings demonstrate that extracts of cruciferous biomass, bark samples, and wool grease together provide complementary active ingredients for the formulation of animal browsing repellent

Graphical Abstract



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Introduction

Sustainable bioproducts engineering seeks to replace synthetic inputs with renewable resources for forestry, agriculture, and environmental management. This thesis aims to develop a sustainable, environmentally friendly, and effective natural browsing repellent formulated from Finnish biomass, targeting large herbivores under boreal conditions. The approach leverages the concept of the circular bioeconomy, a system that emphasises the reuse and recycling of resources and focuses on utilising locally available forestry and agricultural residues, to create bio-based solutions. Natural repellents derived from these sources have the potential to reduce harm caused by moose (*Alces alces*) and roe deer (*Capreolus capreolus*) without relying on chemical pesticides [1,2]. This work explores the extraction and characterisation of bioactive compounds from birch bark, pine residues, cruciferous plants, and sheep wool to meet this goal. The objectives align with recent EU regulatory restrictions on pesticide use, emphasise on non-invasive and environmentally friendly approaches, and reflect the ongoing shift toward sustainable resource utilization [3,4].

Past studies have shown that both synthetic and natural repellents can reduce browsing damage to some extent, particularly when sprayed on young trees and herbivore-encumbered seedlings susceptible to winter herbivory. Synthetic repellents, such as thiram-based compounds and ammonium soaps, have been widely employed but are controversial due to concerns regarding phytotoxicity, persistence in the environment, and regulatory approval under EU Directive 2009/128/EC [3,5,6]. Natural repellents, however, are becoming more popular since they carry a lesser environmental impact. Trico, based on sheep fat, has proved to have strong deterrent effects on deer in field trials, and potassium stearate has proved promising at reducing moose damage to apical leaders [2,7,8].

However, several critical gaps in existing research remain, even as many natural repellent studies focus on a single biomass source and are designed for a single browsing species, thereby limiting their broader utility. Field performance of repellents over the long term, particularly in rain, snow, and bitter cold, remains poorly documented. Seasonal pressures, such as increased browsing pressure due to food shortages in winter, can reduce the deterrent effects, while repeated exposure can lead to habituation, causing the repellent's effectiveness to decline over time [1,9]. Furthermore, there is limited information regarding

the effects of varied extraction techniques on the stability, bioavailability, and deterrent activity of natural compounds, especially when using different biomass sources in combination. These limitations underscore the need for a more integrated and engineering-based approach to natural repellent development, particularly in the context of the boreal forest.

This thesis addresses key gaps related to single-biomass repellents and systematically evaluating how different extraction techniques influence compound stability and activity by developing a natural repellent derived from local biomass available in Finland. The objectives are to: (1) extract bioactive compounds from forest and agricultural materials using Soxhlet, ultrasound-assisted, and solvent extraction; (2) characterize the extracts using UV-Vis spectroscopy, HPLC, FTIR, and GC-MS; (3) compare the chemical profiles of the natural extracts to commercial repellent Trico; and (4) evaluate the environmental impact and feasibility of the repellent. Due to the time limitations, this study is limited to laboratory-scale extraction and characterisation, with no field or long-term ecological testing. The thesis structure comprises the following chapters: Chapter 2 reviews the relevant literature, Chapter 3 covers the materials and methods, Chapter 4 presents the results and discussion, and Chapter 5 concludes with implications and future directions.

1. Literature review

The literature review addresses animal browsing in Finnish forests, its ecological and economic impacts, and the strategies used to mitigate browsing damage. It also discusses repellents, their mechanisms, applications, and limitations, with particular emphasis on natural repellents, their active compounds, extraction methods, and related challenges.

1.1. Animal browsing in Finnish forests

In Finland, harsh winters and forestry operations like clear-cutting lead to increased browsing by large herbivores like moose and roe deer, particularly in young plantations. The herbivores feed on shoots, bark, and twigs of woody plants when ground vegetation is not present [10–12]. The browsing has immense ecological and economic implications, including damage to forest regeneration, reduction in timber quality, and high replanting costs [10,11,13]. Thus, there is a growing interest in the formulation of efficient and sustainable deterrent solutions, particularly those based on biomass-derived chemicals.

Moose (*Alces alces*), roe deer (*Capreolus capreolus*), and other small mammals such as hares and voles are the chief browsing herbivores in Finland. Moose particularly influence the landscape a great deal due to their size, amount of foraging, and change in winter diet towards woody plants, especially in southern and western Finland [10,11,14]. Since the 20th century, the moose population has increased considerably due to more intensive forest management that created superior feeding conditions during early successional stages [11]. Current estimates put that between 200,000–300,000 moose are observed annually, with the 10th National Forest Inventory reporting browsing damage on nearly 990,000 hectares [11,15,16]. Roe deer populations have also grown since the early 2000s, with more than 15,000 individuals by 2003, with significant browsing impacts being localized to southern Finland [17,18].

The most valuable tree species that are impacted by moose and deer are Scots pine (*Pinus sylvestris*), downy and silver birch (*Betula pendula*, *B. pubescens*), Eurasian aspen (*Populus tremula*), and rowan (*Sorbus aucuparia*); all of which have ecological and economic significance [19,20]. Aspen and rowan are especially preferred due to their high palatability, suffering greatly from regeneration losses, while Scots pine, less preferred, is

increasingly browsed when other sources of food are unavailable [10,19,21–23]. Birch generally is subject to frequent attacks by moose, resulting in growth distortion, reduced growth, and increased susceptibility to disease [19,21]. The data presented in the study of Atte *et al.* [23], demonstrates the tree species distribution in Finland in 2013, with forests dominated by pine (~50%), followed by spruce (~30%) and birch and other broadleaves (~20%).

1.2 Impacts of animal browsing

Animal browsing profoundly affects forest structure and composition in Finland, particularly in advanced seedling stands as shown in **Figure 1** Moose as the principal browser, selectively feeds on species such as Eurasian aspen, rowan, and birches which significantly reduces their regeneration potential and allowing subordinate species such as Scots pine increase in abundance and gradually replace more palatable broadleaved species [16,23,24]. Roe deer, while more localized in southern Finland, also contribute to forest dynamics through browsing seedlings of coniferous and broadleaved trees, leading to reduced tree diversity and ecosystem stability [25]. Besides altering species make-up, browsing reduces stem density, slows height growth, and opens the canopy, thereby changing microclimates and habitat quality for other forest organisms [26,27].

Economically, browsing leads to timber defects, growth loss, and tree mortality, causing direct wood quality and quantity losses [20,22]. Management through fencing, repellents, and intensive forest management involves landowner costs in perpetuity [25]. Indirect effects are reduced recreational and aesthetic value of forests, with cascading effects on tourism and recreation- and hunting-based local economies [14]. A further limitation of browsing management lies in species-specific behaviour. Moose and deer differ in browsing patterns, seasonal movement, and sensitivity to stimuli, meaning what works well for one species may fail for another. Additionally, young plants at certain developmental stages, especially during winter season, are particularly vulnerable, and protection strategies must account for these biological windows [10,27].

Climate change exacerbates these problems by altering plant phenology and forage abundance. While higher temperatures can enhance preferred browse species like aspen and birch, competition may compel herbivores to less palatable but economically valuable species like Scots pine [25,26]. Suppression of fast-growing deciduous trees can reduce

forest productivity and carbon sequestration [28]. Browsing also delays canopy closure and exposure of soil, which enhances decomposition, reduces litter accumulation, and diminishes carbon sink capacity [26]. These added ecological and economic pressures highlight the necessity for holistic browsing control methods in forestry.

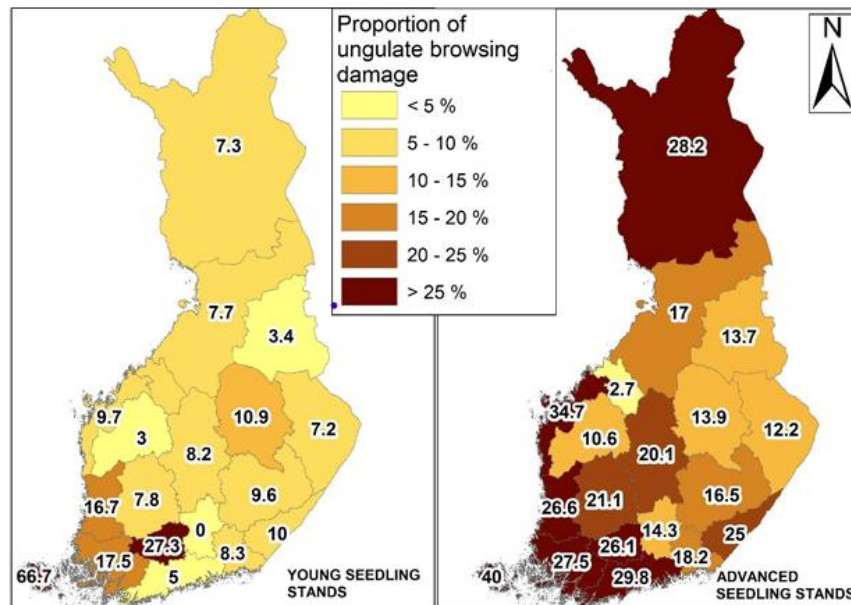


Figure 1. Regional distribution of ungulate browsing damage in Finland, categorized by seedling stand type. The left panel shows damage in young seedling stands, while the right shows damage in advanced seedling stands. Browsing intensity is represented as a percentage of affected area, grouped into five classes from <5% to >25% [27].

1.3 Browsing repellents

Browsing repellents are widely used to reduce herbivory pressure on seedlings and young trees, offering a non-lethal alternative to fencing or population control. This section reviews the main types of repellents, their mechanisms of action, methods of application, and the challenges that limit their effectiveness in forestry practice.

1.3.1 Overview of existing browsing repellents

Repellents offer an environmentally friendly and non-toxic method of lessening herbivore pressure on young plantations. They are employed as a frontline control in Finland and across the Nordic region, especially during winter when other forage is scarce and risk of browsing is high [9]. They are broadly classified into commercial (synthetic) and plant- or animal-derived (natural) products [8]. Commercial repellents are usually synthesized

based on chemicals that are inedible and intimidating to targeted animals. Some of these might include taste deterrents in the form of thiram or bittering chemicals, and chemical-based odours such as putrescent egg solids or ammonium soaps [5]. Natural repellents, on the other hand, extracted from biologically active plant compounds, such as terpenes, sulphur-containing chemicals, or triterpenoids, and sometimes products from predator warning cues like feces or urine [2].

Local natural resources such as spruce bark, Scots pine needles, garlic extracts, and cruciferous vegetable byproducts have been investigated for their repellent properties. These materials are attractive due to their accessibility and lower ecological impact [2,29–31]. Also, waxy natural products such as potassium stearate [8] and lanolin [32].

Potassium stearate is a potassium salt of stearic acid, and it is produced when fatty acids derived from plants are neutralised by potassium hydroxide to obtain a thick and oily substance. Potassium stearate a biodegradable compound and it has shown promising results in deterring cervid browsing in Polish forests [8]. Its pungent and strong odour serves as a good olfactory deterrent against deer. The sticky texture helps the compound adhere to tree bark and shoots, presenting a weak physical barrier [8]. According to Kacprzyk *et al.* [8], the compound was selected as a candidate because stearic acid occurs naturally in plant oils (e.g., shea butter, palm oil), is harmless to the environment, and meets EU policy on low pesticide use and eco-friendly forestry. In field tests, potassium stearate was more effective than the standard synthetic repellent Cervacol Extra PA in protecting silver fir (*Abies alba*) and Scots pine, as the treated trees suffered much lower damage percentages of 18 and 8% respectively (compared to 39 and 23%, respectively).

While synthetic repellents such Ropel and Bonide Deer & Rabbit repellent are often more standardized and widely available than natural ones, they come with concerns over environmental persistence and toxicity. In contrast, natural repellents can be biodegradable and pose fewer risks to non-target organisms, although they sometimes suffer from lower consistency and shorter effectiveness [31,33]. The distinction between commercial and natural products is increasingly blurred, as many newer commercial products now incorporate plant-based ingredients such as garlic extracts and capsaicin to meet environmental standards.

1.3.2 Mechanisms of repellents application

Repellents deter animals by several primary mechanisms; olfactory (odour), gustatory (taste), and, for a few, visual stimuli, as illustrated in **Figure 2**. The olfactory system is particularly important for animals like deer and moose since they base their feeding decisions mostly on odours [14,16,31]. Scent-based repellents include predator-derived substances such as lion feces or putrescent egg solids, which evoke fear or aversion responses in herbivores [34,35]. For instance, the work of Gulsby *et al.*[36] demonstrates that ungulate foraging behaviour is strongly reduced by predator cues, even in the absence of direct contact. This insight underscores the potential of repellents that mimic predator-like signals to deter herbivores.

Gustatory repellents act by making the plant material unpalatable. Common examples include thiram and other bittering agents, which discourage ingestion after initial taste exposure [5]. Naturally derived compounds like capsaicin from hot peppers or the bitterness of betulin in birch bark also play a role in taste-based deterrence. These substances can cause mild irritation or simply create a negative feeding experience that animals learn to avoid [2,37]. Similarly, astringent compounds such as tannins reduce palatability by binding to dietary and salivary proteins, leading to unpleasant mouthfeel and reduced digestibility. Studies reported that tannin-containing plants are always avoided by browsing ungulates, which speaks well of their prospects as natural deterring agents [38].

Visual repellents are less commonly used but may contribute to overall deterrent effects when combined with olfactory cues. Some studies suggest that bright coloration or reflective surfaces may startle or confuse herbivores, reducing their browsing activity. For example, the white bark of birch has been hypothesized to act as a visual deterrent due to its association with unpalatable chemical content [1,2]. According to Ireland & Ruxton [1], the white colour of birch bark caused by the surface deposition of ultraviolet-reflecting chemicals such as betulin can act as a visual deterrent against mammalian herbivores. Their findings indicated that white birch bark reflects not only ultraviolet (UV) but also visible light and would trigger avoidance behaviour in bark-stripping animals such as deer and hares. They also showed that herbivores removed bark preferentially from darker substrates over natural white birch, suggesting whiteness could be a chemical defence-free visual

warning signal. Deer vision complies with this interpretation because deer are dichromatic and sensitive in the blue–green range and can see ultraviolet light, unlike humans [39,40].

In practice, many repellent formulations combine multiple mechanisms. Commercial products like Trico, which relies on sheep fat, offer strong scent deterrents and have been successfully applied in forests across Europe as well as United States [7]. Combining mechanisms is particularly useful in addressing habituation, where animals become desensitized to a single stimulus over time.

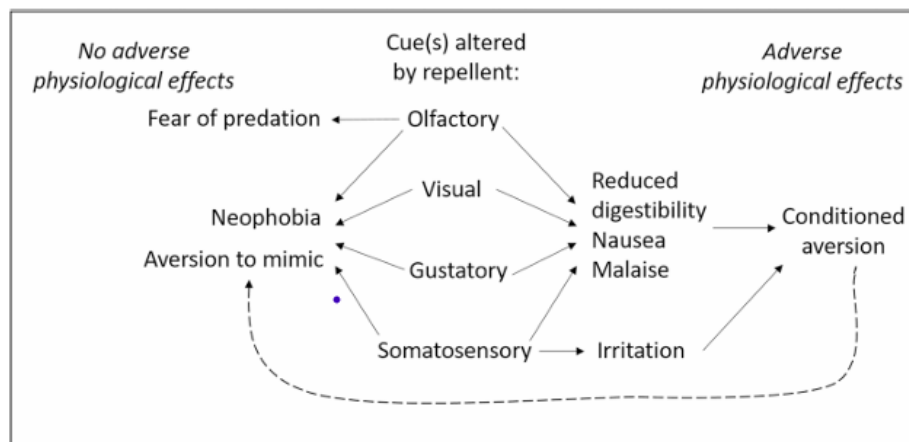


Figure 2. Conceptual model illustrating the mechanisms by which repellents deter herbivores through olfactory, visual, gustatory, and somatosensory cues [41].

1.3.3 Methods of repellent application and associated challenges

Repellent application methods vary widely depending on the formulation and the setting. The most common delivery systems include hand spraying, painting, fogging, or applying granules or tablets at the base of plants [8]. In forest contexts, spraying and painting are particularly common, allowing direct treatment of the most vulnerable plant parts, such as apical buds and stems [2]. However, these methods require intensive labour effort and are often hard for large-scale production.

Environmental conditions heavily influence application efficacy; rain, snow, and UV exposure can degrade repellent residues, especially for volatile or water-soluble compounds like allicin and terpenes [42]. This results in the need for frequent reapplication, increasing both labour costs and environmental exposure. In addition, application in winter presents unique challenges in Finland, where snow depth, frozen ground, and limited accessibility to

forest plots complicate equipment use. Manual application may require snowshoeing or mechanized support, which adds to the operational burden [8].

Some promising solutions to such environmental conditions include slow-release matrices or encapsulated compounds that adhere better to plant surfaces and resist weathering [43]. According to Boh *et al.* [43], the commercial odorant-based deer repellent Daphne, consisting of a mixture of fragrances such as vanillin, citronellol, and heliotropin, was encapsulated successfully with melamine–formaldehyde polymers to enhance stability and persistence. The formulation was even further augmented by adding polyvinyl alcohol (PVA) and acrylate latex as binders, which improved adhesion to plant substrates and water resistance. During winter field trials, microencapsulated repellent significantly reduced roe deer (*Capreolus capreolus*) and European hare (*Lepus europaeus*) browsing damage by more than 70 days.

Further research has explored encapsulation of essential oils such as limonene, camphor, and thymol by methods including spray-drying, ionic gelation, and coacervation, resulting in improved long-term efficacy and field stability [44,45]. Compared to earlier encapsulation systems based on synthetic polymers such as melamine–formaldehyde, these newer systems are based on biodegradable, food-grade materials such as garlic that are more environmentally benign and more suitable for sustainable repellent products.

Another major factor that limits the choices between natural and synthetic repellents is the cost. While synthetic repellents may offer longer duration of protection and higher active ingredient concentrations, their higher cost and potential environmental effects, chemical runoff or nonbiodegradability, may limit their widespread use, particularly in environmentally sensitive or low-income areas [31]. In contrast, natural repellents are generally lower in cost and more biodegradable because they are metabolically broken down and have lower risks to human health and the environment. However, they may have to be used more frequently and with higher doses to be as effective as synthetics, thus theoretically increasing usage and labour costs in the long run [46].

1.3.4 Effectiveness and limitations of current repellents

Field evaluations of repellents often reveal mixed results. While many formulations show short-term success, long-term efficacy is frequently compromised by animal habituation, environmental degradation, and inconsistencies in application [6]. In Finnish

and Swedish forests, spruce bark extract applied to Scots pine reduced apical leader browsing by more than 50%, but lateral shoot browsing remained a concern [2]. Similarly, potassium stearate offered significant protection but washed off easily in rain and snow, limiting its persistence [8].

Repeated exposure can also lead to reduced deterrence; animals may learn to tolerate or ignore scent- or taste-based stimuli if they pose no real danger. This is especially problematic for synthetic compounds used season after season, leading to declining effectiveness over time [1]. Moreover, toxicity and non-target effects remain concerns, particularly for synthetic formulations. Environmental persistence can interfere with soil microbiota or affect beneficial insect populations [19,47]. Different repellents have been studied depending on the targeted plant species and herbivore browsers, **Table 1** summarises some of the existing repellents, focusing on chemical and natural repellents derived from different starting materials, including their application mode and method as well as their effectiveness.

Table 1. Overview of repellent types, application strategies, and their effectiveness against browsing by deer and moose, as reported in previous studies

Repellent type	Mode of application	Application method	Targeted species	Notes on effectiveness	Reference
Pine tar, Top Dendrocol	Aversion, taste	Spray (manual hand-tricker pump)	Moose	Lower damage to treated pine saplings, no difference between the treatments.	[29]
Tree Guard (Bitrex), Eutrofit (animal blood), hot sauce (capsaicin)	Taste, odour	Spray (manual knapsack-type sprayer)	Fallow deer (<i>Dama Dama</i>)	Tree Guard and Eutrofit significantly reduced browsing; Hot sauce less effective.	[48]
Norway spruce bark extract	Odour	Spray	European moose (<i>Alces alces</i>)	In trial 2 at a concentration of 5.0%, the browsing of apical leaders dropped to 4.9% in the treated trees.	[2]
Egg albumen, 5% methionine in lime, bake's soy flour, complete milk protein, hydrolysed casein.	Taste aversion	Spray with agricultural sticker	Black-tailed deer (<i>Odocoileus hemionus columbianus</i>)	Hydrolysed casein at 8%-12% concentrations reduced deer browsing, however methionine-containing treatments showed good efficacy as well.	[30]
Hot Sauce (Capsaicin), Big Game Repellent Powder	Irritant (trigeminal), taste	Spray (6.2 % with anti-transpirant)	Black-tailed deer (<i>Odocoileus hemionus</i>).	6.2% hot sauce solution reduced deer browsing to redcedar seedlings (<i>Thuja plicata</i>) however its efficacy declined after 2 weeks as opposite to Big Game Repellent powder which maintained the deterring effect the whole study period.	[37]
Trico (sheep grease), Plantskydd (dried blood)	Odor	Sprayed to drip point on dry foliage	White-tailed deer (<i>Odocoileus virginianus</i>)	Trico effectively reduced browsing on yaw shrubs for up to 12 weeks compared to Plantskydd. However, during intense browsing pressure, both repellents failed.	[7]
Potassium stearate, Cervacol Extra PA repellent	Odor, taste	Applied manually (greasy consistency)	Red deer (<i>Cervus elaphus</i>) and roe deer (<i>Capreolus capreolus</i>)	Potassium stearate provided the best protection to mature Scots pine and silver fir trees and in some cases was more effective than Cervacol Extra PA.	[8]

1.4 Natural repellents

Natural repellents have gained increasing attention as alternatives to synthetic chemicals, with potential for effective browsing deterrence in a reduced environmental impact. This section reviews the common active compounds found in natural sources, methods used in extracting these compounds, and formulation difficulties related to stability and efficacy.

1.4.1 Common active compounds in natural repellents

Native and cultivated plant species in Finland, such as birch, Scots pine, and cruciferous plants, produce a range of bioactive compounds [49]. These same plants have also been shown to deter herbivores and hold considerable promise for the development of natural repellents. Notably, they contain secondary metabolites such as triterpenoids, terpenes, flavonoids, and sulphur-containing molecules, which can act on the sensory systems of browsing animals by producing unpleasant tastes, strong odours, or irritation, ultimately discouraging feeding [31,50].

1.4.1.1 Extractives found in Birch bark

Birch bark contains pentacyclic triterpenoids like betulin which constitutes up to 20–30% dry weight in the external bark layer and sometimes even up to 40% [51]. However, the chemical characteristics of birch vary depending on geographical location, climate, growth stage, and birch sections [52,53]. Almost all extracts include betulin which is responsible for the white colour in birch bark [51]. Aside from the antifungal and antimicrobial properties of such compounds ((e.g., betulin, betulinic acid, lupeol, and phenolic acids), they also have an extremely bitter taste, therefore stand as a potential repellent based on taste [51,52]. According to Bergvall *et al.* [52], the feeding study demonstrated that pellets intended for cervides treated with concentrations of 1% of birch extract or higher had a repellent effect against the consumption by fallow deer, regardless of the extraction method. In contrast, lower concentrations did not significantly reduce pellets consumption by deer.

1.4.1.2 Extractives found in Scots pine

Scots pine bark and needles are recognised for their high extractive content. The overall extractive percentage in Scots pine biomass varies according to component, with

typical values given as follows: needles 40%, bark 25%, stump 19%, branches 17%, roots 13%, and stem wood 3–5% [54,55]. According to studies, typical levels vary between 1.0% and 6.8% [50]. Turpentine, a solvent and source of synthetic platform chemicals like α -pinene, is made from pine oleoresin, which contains terpenoids generated from isoprene units [56]. The primary terpenoids found in pine oleoresin are monoterpenes (C10), sesquiterpenes (C15), and diterpenoid resin acids (C20), all of which possess valuable commercial significance [56,57]. Volatile terpenes, which are frequent natural wood extractives, often exist at concentrations of 0.02–2% and contain principally monoterpenes α -pinene, β -pinene, Δ^3 -carene, and limonene [58,59]. They are responsible for the distinctive wood odour and are mostly emitted during different industrial operations such as debarking, chipping, steaming, mechanical refining, and chemical pulping of softwood[42]. These compounds are attributed with the pine's intense resinous odour, which has been shown to be an olfactory deterrent to moose and deer browsing.

1.4.1.3 Extractives found in Garlic

Aside from woody plants, different cultivated plant materials have been studied including garlic (*Allium sativum*). Garlic is composed mainly of water (60–65%), followed by carbohydrates (28–30%), proteins (2–6%), organosulfur compounds (~2.3%), fibre, fatty acids, phenols, and trace minerals (~1.5%), and amino acids (~1.2%) [60,61]. Furthermore, the group of sulphur compounds found in garlic has been demonstrated to include several active compounds, such as allicin, alliin, S-allylcysteine, diallyl disulphide, diallyl trisulfide, diallyl sulphide, and ajoene [62]. Such compounds are enzymatically synthesized when the plant tissue is damaged which gives garlic its pungency and irritant as well as antimicrobial properties [63]. Different studies have incorporated garlic extracts into insect repellents. However, according to the study conducted by Curtis *et al.* [64] where they tried to select ten plant candidates to develop a natural repellent against voles, the garlic-based repellent proved somewhat effective, but far less so than top performers such as pachysandra and boxwood, which decreased vole eating by up to 85% at low concentrations.

1.4.1.4 Extractives found in cruciferous vegetables

Similarly, cruciferous vegetables such as cabbage, red cabbage, and broccoli are highly valued for their bioactive components, glucosinolates (GLSs), and sulforaphane (SFN) [65,66]. Even though not yet incorporated in repellent development, brassica vegetables have a high concentration of GLS, which varies depending on genotype, variety,

cultivation circumstances, developmental stage, plant tissue type, and postharvest handling conditions [67]. GLSs are hydrolysed into isothiocyanates such as sulforaphane upon tissue disruption [68]. The breakdown products are bitter and toxic to insects and certain mammals. Sulforaphane was demonstrated to retard herbivore feeding under laboratory conditions, particularly when the plant extract has high enzymatic activity [68]. Broccoli and cabbage in crucifers generally have higher glucosinolate content than garlic, making them attractive sources for natural repellents [69].

1.4.1.5 Extractives found in sheep wool grease

Sheep wool grease, commonly known as lanolin, is a waxy, water-repellent substance secreted by the sebaceous glands of wool-bearing animals, especially sheep. It accumulates on wool fibers as a natural protective barrier, preventing dehydration of the fleece and helping to repel environmental contaminants such as rain, dust, and microbes. Chemically, lanolin is distinct from fats and oils because it does not contain glycerol; it is classified as a wax due to its structure, which consists primarily of esters formed from a wide range of fatty acids and long-chain alcohols. Key components of lanolin include cholesterol, isocholesterol, aliphatic alcohols, and hydroxy acids, which collectively contribute to its moisture-sealing and film-forming properties [32,70]. These compounds are amphiphilic in nature, allowing lanolin to form stable emulsions with water and adhere well to surfaces [32,71], which is a valuable trait for use in forestry and agricultural applications where long-lasting coverage is critical. From an ecological and practical perspective, lanolin offers significant potential as a mammalian repellent. Its persistent greasy texture and strong odour may act as a physical and olfactory deterrent, creating an unfavourable surface on plant shoots and foliage that discourages feeding by browsing animals.

1.4.1.6 Main components of sheep fat

Sheep fat consists mainly of triglycerides in the form of fatty acids (e.g., palmitic acid, stearic acid, and linoleic acid) [72]. In a documented field study, the commercial repellent Trico, which contains 6.4% sheep fat as its active ingredient, was shown to significantly reduce deer browsing on ornamental yews. In this multi-site study by Curtis *et al.* [7], Trico provided effective protection for up to 12 weeks in three out of four suburban locations, even under challenging winter conditions with snow cover and freezing temperatures. The product was applied as a foliar spray and retained efficacy without reapplication for nearly three months, indicating that sheep fat-based formulations may offer

a practical solution for winter protection of vulnerable plantings in boreal regions like Finland.

Figure 3 illustrates the chemical structure of two representative bioactive compounds investigated in this study. Panel (a) shows sulforaphane, an isothiocyanate derived from glucosinolates in cruciferous biomass and Panel (b) presents betulin, a pentacyclic triterpenoid abundant in birch bark.

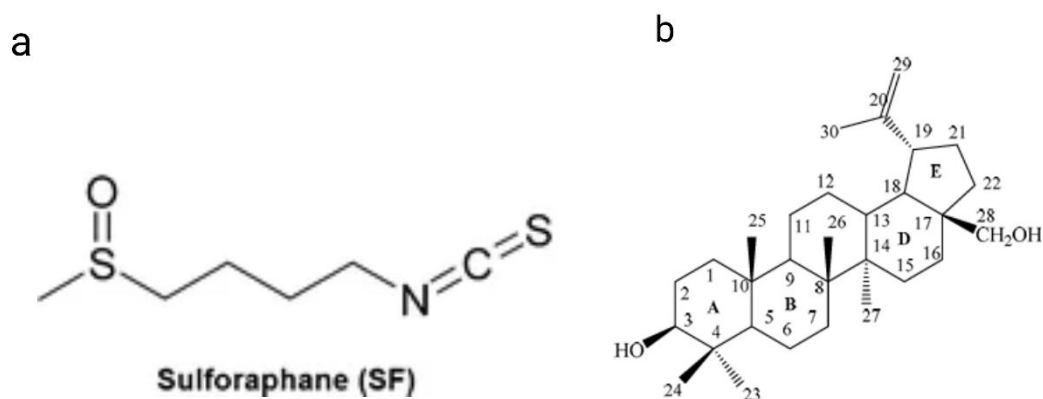


Figure 3. Chemical structure of Sulforaphane (a) and Betulin in Birch bark (b).

1.4.2. Extraction methods for active compounds

The method for extracting active compounds has a strong correlation with their yield, stability, effectiveness, and even their mode and method of application [73]. Different extraction techniques must be carefully selected based on factors such as the thermal sensitivity, volatility, and solubility of the target molecules, as well as operational parameters including solvent type and volume, energy consumption, scalability, and overall environmental impact. A well-chosen extraction method not only preserves the functional integrity of bioactive compounds but also supports the development of safer, more sustainable formulations [74–76].

1.4.2.1 Extraction methods of betulin in birch bark

Betulin is extracted from birch bark by several methods including simple extraction using organic solvents such as aqueous alkali-ethanol mixture, butan-1-ol, chloroform/dichloromethane/methanol mixture, dichloromethane, ethanol, ethyl acetate and its mixtures with ethanol and water, limonene, methanol, *n*-heptane, *n*-hexane, petroleum ether, propan-2-ol, toluene and others [52,77–79]. However, these solvents are relatively

varied in their environment and health impact. Ethanol, ethyl acetate, limonene, and propan-2-ol are generally considered environmentally friendly due to their biodegradability, lower toxicity, and renewable source. On the other hand, solvents such as chloroform, dichloromethane, *n*-hexane, and toluene are of concern due to their toxicity, volatility, and environmental persistence. Thus, solvent selection must prioritise low toxicity, renewability of feedstock, and low environmental footprint as advocated by safer chemical design and sustainable processing [80].

Other extraction methods for betulin involve conventional Soxhlet extraction, 3–50 g of bark undergoes 12 cycles using ethanol or a mixture of ether-acetone at 40–60 °C, giving a lipophilic fraction with high betulin content [78,79]. This procedure is common and efficient when bark is powdered dry to expose a wide surface area, nonetheless, it is slow and solvent intensive but easy and straightforward [78,79]. Other methods include pressurized liquid extraction using ethanol at 120 °C and 50 bar in an accelerated solvent extractor which proved to recover around 26 wt% betulin in 15 min [81]. Also, different studies have presented supercritical CO₂ extraction as an eco-friendly and effective method for separating lipophilic chemicals from biomass, acting as a sustainable alternative to standard solvent extraction in different biorefinery applications. It is usually performed at 300–400 bar and 50–60 °C for 2–3 hours, with subsequent organic solvent washes, affords a solvent-free extract that preserves heat-sensitive antioxidants alongside betulin [52,79]. Yet, compared to conventional solvent extraction methods, supercritical CO₂ extraction is far more expensive due to its high-end equipment and operational requirements, particularly the pressure-proof systems and energy-intensive compression.

1.4.2.2 Extraction methods for pine bark compounds

In studies on pine, various extraction techniques have been applied to isolate valuable compounds such as terpenoids and acidic/neutral fractions. Zommere *et al.* [75], employed methods including microwave-assisted extraction with 5% NaOH at 150 °C, ultrasound-assisted extraction using multiple solvents like NaOH, hexane, and ethanol, maceration at boiling point with solvents such as acetone and ethanol, aqueous-alkaline extraction, and supercritical CO₂ extraction at pressures up to 350 bar. Similarly, Bertaud *et al.* [42] investigated terpenoid extraction using conventional Soxhlet reflux with acetone and acetone/cyclohexane mixtures, accelerated solvent extraction with *n*-hexane and acetone/water under high temperature and pressure, steam distillation via a Clevenger

apparatus, and supercritical CO₂ extraction at 60 °C and 20–30 MPa, sometimes with ethanol as co-solvent, measuring extraction efficiency by mass loss. Notably, Soxhlet extraction conducted on fresh, ground samples using successive acetone and acetone/cyclohexane (1:9) treatments provided the most representative quantification of volatile terpenes, resins, and fatty acid-type compounds [42].

1.4.2.3 Extraction methods for sulphur-containing compounds from garlic

Sulphur-containing compounds in garlic can be extracted using traditional methods such as Soxhlet extraction and distillation, as well as modern green techniques such as ultrasound-assisted extraction (UAE), microwave-assisted extraction, and supercritical fluid extraction [74]. While older techniques are preferred due to their simplicity and compound stability, integrating them with current technology improves efficacy, with time, temperature, and solvent optimisation critical for maximising extraction quality and efficiency [74]. Water is a preferred solvent due to its environmental safety, selectivity, and suitability for food and pharmaceutical use, with fresh garlic water extracts showing highest allicin (AC) content (42.74–50.79 µg/ml) and measurable sulphur compounds like methyl methanethiosulfonate, allyl sulphide, and diallyl disulphide, though concentrations vary by variety [82]. UAE with water is especially effective in extracting thiosulfinates (TS), providing rapid, low-temperature processing that preserves heat-sensitive compounds and yields high TS recovery (6.42 µmol/g dw) [73], outperforming organic solvents like ethanol, which generally yield lower AC levels despite good TS extraction [82,83]. The solvent polarity also influences extraction of other compounds such as phenolics, with water extracts typically yielding fewer polyphenols than organic solvents [74].

1.4.2.4 Extraction methods for sulphur-containing compounds from crucifers

Likewise, for sulforaphane (SFN) and glucosinolates (GLS) extraction from brassica vegetables, there exists conventional methods such as solvent extraction with methanol or methanol-water mixtures for GLS preservation and enzymatic hydrolysis to SFN under controlled pH and temperature conditions to avoid unwanted byproducts such as nitriles [84,85]. Other novel techniques, such as high-pressure processing, microwave-assisted extraction, high voltage electrical discharges, and supercritical fluid extraction, are being developed to improve efficiency, reduce solvent and energy consumption, and improve the quality and yield of different isothiocyanates (ITCs) compounds including SFN [84]. These methods optimise factors such as pressure, temperature, and energy input to maximise

extraction, but newer approaches such as fermentation-assisted extraction and ultrasound-assisted dispersive filter extraction provide novel, cost-effective alternatives [84].

According to different studies, UAE serves as an increasingly popular eco-friendly method that reduces solvent use, energy, and time [86]. UAE equipment frequencies range from 20 to 50 kHz, with power varying from 100 to 500 W, typically using aqueous solvents such as water alone or combined with ethanol, methanol, or acetonitrile [87]. Liu *et al.* [88] found a water-to-material ratio of 1:10 more effective than 1:50 ethyl-to-acetate ratio for SFN extraction. Solid-to-liquid ratios in most studies range between 1:2 and 1:50 [20]. Martínez-Zamora *et al.* [89] employed a UAE extraction with particle size <56 µm and distilled water as solvent, testing solid/liquid ratios of 1.25 and 2.25 g/mL, temperatures of 25, 40, and 55 °C, and times from 0 to 20 minutes on broccoli florets and leaves and found out that temperatures below 30 °C favour GLS and SFN extraction, contrasting with higher temperatures (>45 °C) used for phenolics.

1.4.2.5 Extraction methods for wool grease

Traditional extraction of wool grease involves using Soxhlet apparatus and organic solvents such as dichloromethane. Although good, large amounts of toxic solvents are usually used, beside longer extraction times (around 4h). Also, toxic solvents raise severe environmental and health issues. To address these issues, alternative solutions were proposed including the usage of less toxic solvents (e.g. ethanol, acetone etc.) as well as environmentally friendly methods, including supercritical fluid and microwave-assisted extractions [70,90,91]. Such approaches help reducing the amount of solvent needed and extraction times. López-Mesas *et al.* [92] compared supercritical fluid and microwave-assisted extraction alongside automated Soxhlet extraction and confirmed that while microwave-assisted extraction allows for multiple sample processing compared to other methods, supercritical fluid extraction still stands out due to using CO₂ (inert, non-toxic, and non-polar) especially when combined with solvents like acetone to adjust polarity and improve its efficiency [90,91]. However, such approaches require sophisticated materials and increase the costs, thus, Soxhlet extraction may be preferred due to its simplicity and accessibility [70].

1.4.3. Physico-chemical stability and repellent formulation challenges

A wide range of factors affect repellent effectiveness such as compound solubility, adhesion to plant surfaces, and vulnerability to environmental factors. Alcohol carriers improve penetration but are volatile and may harm non-target organisms. Water-based formulations are safer but usually need stabilizers or UV protectants. In forestry, where manual reapplication is difficult, slow-release and wash-off resistant products are critical. [93].

Most natural repellent compounds are chemically reactive, volatile, or sensitive to light, posing challenges for formulation and field use. For example, betulin is more chemically stable than terpenes or allicin but has poor solubility and tends to crystallize on surfaces, making even application difficult [51,52,74]. Therefore, researchers are exploring emulsifiers and nanoparticles to improve its dispersion [77]. Similarly, terpenes are extremely volatile compounds which accounts for their temporary effectiveness, yet their rapid evaporation under outdoor conditions limits their persistence [42].

When it comes to SFN and similar ITCs, which degrade under heat, pH changes, and enzymatic activity, so encapsulation in biodegradable carriers is used to preserve their activity and reduce reapplication [68,89]. In addition, allicin in garlic is highly reactive and will begin degrading following a span of seconds when exposed to air or high temperature. Studies have shown that over 50% of allicin may degrade during 15 minutes at 60°C, and even at lower temperatures it will require protective formulations to be efficient [63]. For this reason, microencapsulation in starch-based or lipid carriers has been proposed as a way of stabilizing allicin and enhancing its shelf life [94].

Lanolin stands out for its physical and chemical resilience. Unlike many natural oils, it resists wash-off and degradation from rain, snow, and UV exposure due to its high melting point and hydrophobic nature [32,70]. This makes it a promising carrier or base in repellents, especially when combined with other natural actives like terpenes or sulphur compounds to boost effectiveness and reduce the need for frequent reapplication. Regardless, extensive studies are required for its conformity to EU's guidelines.

1.4.4. Ethical considerations in repellent development

The ethical development of natural repellents in Europe and, in this case, Finland is connected head-on with forestry and land use sustainability principles. Sourcing raw materials is one of the most important concerns. Precious bioactive substances are found in Birch bark, Scots pine needles, and other wood by-products, but they must be harvested responsibly. In Northern European countries, utilizing residual biomass from forest work, rather than live trees, is not only an eco-friendly but also a superior moral approach. Studies have shown that the chemical composition and extractive yield vary by season and post-harvest treatment, which emphasizes careful planning and sustainable operation in raw material cutting [79,95]. This aligns with overall biorefinery goals in Finland to minimize waste and enhance the value of harvested biomass.

European Union legislation also dictates ethical considerations in repellent preparation. Regulations such as Directive 2009/128/EC and Regulation (EC) No. 1107/2009 dictate the restraint of synthetic pesticide use and the promotion of a transition to biologically based alternatives [3,4]. As such, naturally occurring chemicals such as potassium stearate with high breakdown rates and low toxicity risk exposure levels have been authorized for application on forest and agricultural applications [8]. In contrast, natural repellents composed of biodegradable, plant-based materials are not likely to persist in the environment or harm non-target organisms. However, ensuring ecological safety through testing for impact on beneficial organisms (e.g. pollinators and soil microbes...etc) is a vital step.

2. Aims and scopes

This study aims to develop a natural repellent to protect Finnish forest and garden vegetation from browsing by moose (*Alces alces*) and roe deer (*Capreolus capreolus*). This study is focused on conditions specific to Finland and targets the two main browsing species. All development and testing are limited to laboratory and small-scale field settings.

To do this, the specific scopes of this work include:

1. Extraction and purification of active compounds from birch, pine, wool grease, and cruciferous crops.
2. Characterisation of the chemical properties of the extracts to identify the active compounds.
3. Comparison of natural extracts to the commercial product Trico.
4. Evaluation of the environmental impact and feasibility of the repellent.

3. Methodology

This section describes the experimental procedures followed to study the extraction, characterisation, and evaluation of bioactive compounds from cruciferous biomass, birch and pine bark, as well as sheep wool.

3.1 Chemicals and equipment

Commercial chemicals and reagents used in this study are listed in **Table 2**, including solvents, reagents, and analytical standards used in the extraction and characterisation procedures. Meanwhile, equipment used for extraction trials, sample preparations, and analytical characterisations of plant- and wool-based extracts are listed in **Table 3**. The cruciferous biomass and reference products are presented in **Table 4**.

Table 2. List of chemicals used in the experiments.

Chemical / Reagent	Specification	Laboratory	Country
Ethanol (Etax A)	≥94.0%	Anora Group Oy	Finland
Folin–Ciocalteu reagent	2 M (with respect to acid)	Sigma-Aldrich	Switzerland
Gallic acid	≥99%	Sigma-Aldrich	China
Sodium carbonate (Na ₂ CO ₃)	≥99.0%	VWR Chemicals	Germany

Table 3. List of equipment employed in the experiments.

Instrument / Equipment	Model / Specification	Manufacturer	Country
Cutting mill	SM 300, 3000 W, 220–230 V, 50/60 Hz	Retsch GmbH, Germany	Germany
Soxhlet extractor condenser	Cone NS 45/40, 100–250 mL	Lenz Laborglasinstrumente, Germany	Germany
Rotary evaporator + vacuum pump	Hei-VAP with Hei-VAC Vario Control, 1.7 m ³ /h, 2.0 mbar	Heidolph Instruments GmbH, Germany	Germany
Ultrasonic bath	USC300TH, 45 kHz, 80 W, heater 200 W	VWR International, Malaysia	Malaysia
UV-Vis spectrophotometer	Evolution 500, 190–1100 nm	Thermo Electron Corporation, UK	UK
FTIR spectrometer	Frontier FTIR with diamond ATR, 400–4000 cm ⁻¹ , 4 cm ⁻¹ resolution	PerkinElmer, USA	USA

Glass microfiber filters	GF/A, 70 mm diameter, 1.6 μm pore size	Whatman / Cytiva, China	China
Moisture analyzer	VWR Moisture Analyzer (with halogen heating unit)	VWR International Ltd, UK	UK

Table 4. List of purchased products for the experiments.

Product	Type/Use	Brand/Producer	Origin
White cabbage (Keräkaali)	Vegetable biomass	Kotimaista (SOK)	Finland
Red cabbage (Punakaali)	Vegetable biomass	Kotimaista (SOK)	Finland
Broccoli	Vegetable biomass	Kitto's Organic	Spain
Trico Garden	Commercial repellent (reference)	Kwizda Agro GmbH, Austria	Finland
Sheep fat cubes (pet food)	Animal fat (reference)	"Basic" Superfood	Netherlands
Lanolin cream (HPA®)	Wool wax (reference)	Lansinoh Laboratories	USA / Germany

3.2 Sample preparation

All the raw materials used in this study were first prepared to yield consistency, reproducibility, and compatibility with subsequent extraction procedures. Fresh cruciferous vegetables were immediately shredded upon purchase and stored at 4 °C until they were used. Sheep wool and wood biomass samples were stored at –20 °C prior to treatment. To achieve maximum extraction efficiency, solid samples were ground to reduce particle size and increase surface area, thereby facilitating solvent access. The individual preparation processes for cruciferous biomass, wood bark, and sheep wool are dealt with in the following subsections.

3.2.1 Cruciferous biomass

Fresh broccoli (*Brassica oleracea* var. *Spain*), white cabbage (*B. oleracea* var. *capitata* f. *alba*), and red cabbage (*B. oleracea* var. *capitata* f. *rubra*) were purchased from a local supermarket (Prisma, Lahti, Finland). The vegetables were utilized shortly after purchasing without initial washing or drying. Broccoli florets as well as pieces of the stems were utilized, whereas cabbage and red cabbage were utilized as entire heads. Samples were manually chopped with a knife and homogenized afterwards in a Philips HR1393/00 blender (Philips, Netherlands; capacity 0.7 L, 450 W motor, stainless steel knife, plastic

bowl). Homogenized biomass was directly subjected to ultrasound-assisted extraction (UAE). Remaining material was stored unprocessed (without chopping and blending) at 4 °C for future use. **Figure 4** Illustrates the sequential processing steps before UAE.

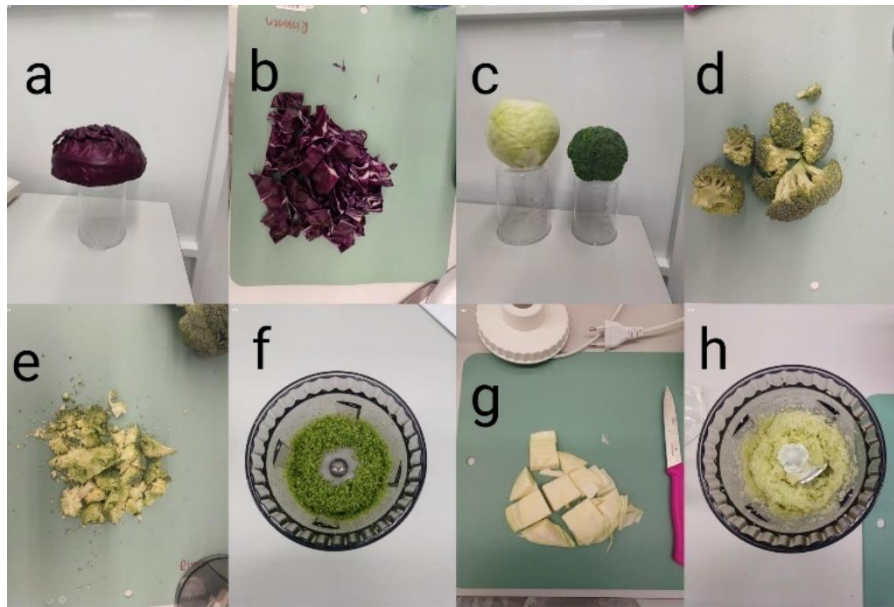


Figure 4. Preparation steps of cruciferous biomass prior to extraction. (a) Whole red cabbage, (b) chopped red cabbage, (c) whole cabbage and broccoli, (d) chopped broccoli, (e) chopped broccoli, (f) blended broccoli, (g) chopped white cabbage, (h) blended white cabbage.

3.2.2 Wood biomass

Birch (*Betula pendula*) and pine (*Pinus sylvestris*) bark samples were kindly donated by Koskisen Oy (Järvelä, Finland). The bark was air-dried to a constant weight (19 days), after which it was ground using a cutting mill (SM 300, 3000 W, 220–230 V, 50/60 Hz; Retsch GmbH, Germany). The bark material was sieved to have a uniform particle size (<4 mm for pine bark; <4 mm and <1 mm fractions for birch bark). Processed bark biomass was stored in sealed bags at –20 °C until the time of Soxhlet extraction. **Figure 5** Illustrates the sequential preparation steps for bark biomass.

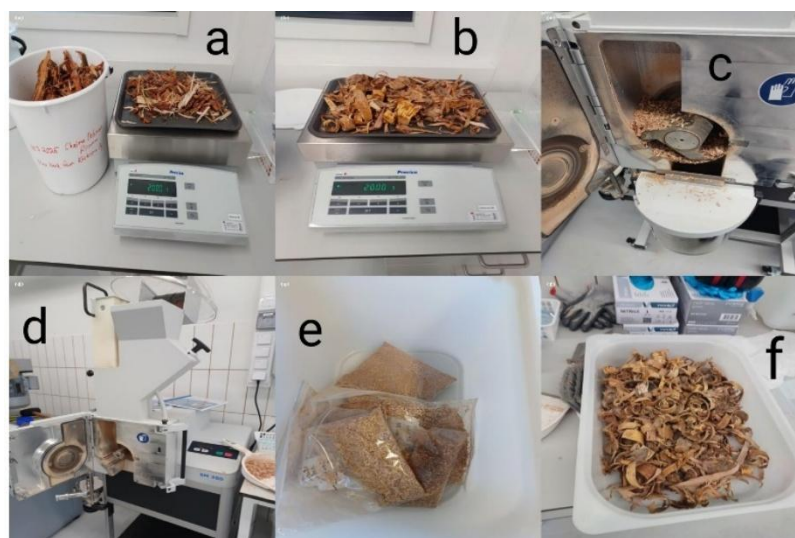


Figure 5. Preparation of bark biomass prior to extraction. (a) Pine bark samples before drying, (b) birch bark samples before drying, (c) bark grinding inside the cutting mill, (d) SM 300 cutting mill, (e) ground bark samples packed in sealed bags, (f) birch bark after air drying.

3.2.3 Sheep wool

Sheep wool samples were kindly donated by Pirtin Kehräämö (Mikkeli, Finland) and included wool samples from Texel breed and Finnsheep. Wool was manually cleaned to eliminate plant matter residues and outer dirt but not washed with water or detergent. Hand-cleaned fibers were separated manually into smaller pieces to provide more surface area and facilitate easier solvent penetration during Soxhlet extraction. Prepared wool samples were used directly in extraction experiments.

3.3 Extraction procedures of different biomasses

Different extraction processes were employed for the separation of bioactive compounds and lipophilic molecules from cruciferous biomass, wood bark, and sheep wool. Ultrasonic-assisted extraction (UAE) was applied to fresh cruciferous material since it can extract thermolabile compounds with gentle conditions. Soxhlet extraction was performed for most of the wood biomass and wool in a way that allows exhaustive extraction with continuous reflux of solvent. In addition, solvent immersion as a simple, static extraction technique was used for birch bark to compare recovery with Soxhlet extraction. Two solvents were used throughout experiments: distilled water and ethanol (ETAX A). Following extraction, all samples were filtered, concentrated, and stored under controlled

conditions prior to analysis. Specific procedures for each method are detailed in the following subsections.

3.3.1 Ultrasonic-assisted extraction of cruciferous biomass

Ultrasonic-assisted extraction was performed following the general procedure of Martínez-Zamora *et al.* [89], with modifications. Freshly prepared cruciferous biomass was extracted with a biomass-to-solvent ratio of 8 g biomass to 100 mL solvent. Two solvents were employed: distilled water and ethanol. Extraction was performed at 25 °C and 50 °C for 20 min in an ultrasonic bath. In addition, a freezing pre-treatment was also done in which 8 g of homogenized biomass was stored at -20 °C for 24 h prior to extraction with distilled water at 25 °C and 50 °C under otherwise identical conditions. Due to time constraint, frozen biomass was not extracted with ethanol. The experiments were performed in triplicates and conditions of extraction were recorded accordingly. **Figure 6** presents the ultrasonic extraction process and examples of broccoli and red cabbage extracts, along with the biomass after freezing pretreatment.

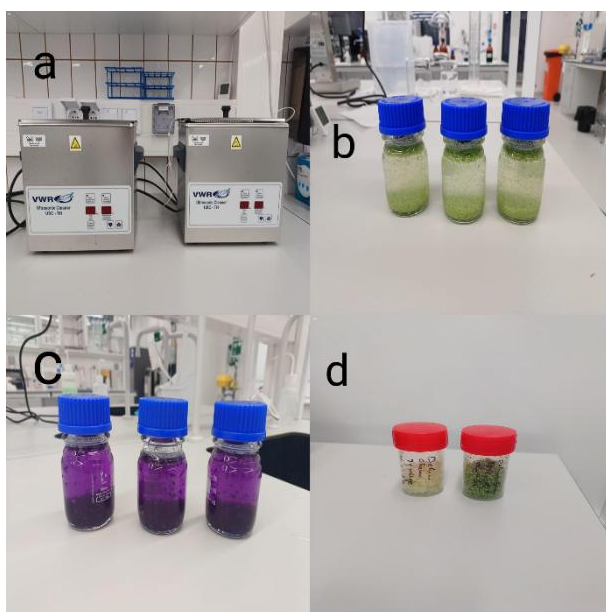


Figure 6. Cruciferous biomass extracts and experimental setup. (a) Ultrasonic bath used for ultrasound-assisted extraction, (b) Broccoli extracts after UAE in water, (c) Red cabbage extracts after ultrasound-assisted extraction in water, (d) Cruciferous biomass after freezing pretreatment.

3.3.2 Soxhlet extraction of wood biomass and sheep wool

Soxhlet extraction of birch bark, pine bark, and sheep wool samples was carried out using ethanol (ETAX A) as solvent. For birch and pine barks, the method of Bergvall *et al.* [52], with minor modifications was followed; 15 g of pre-treated biomass (<4 mm particle size) was placed in a cellulose thimble and extracted with 300 mL ethanol. Extraction was extended for 12 cycles, which amounted to approximately 6 h, with a duration of 25–30 min per siphoning cycle. For pine bark, the method of Bertaud *et al.* [42] with minor adjustments was followed; 20 g of pre-treated biomass (<4 mm) was extracted with 300 mL ethanol for 10 cycles (~5 h), under the same cycle duration.

Sheep wool (Texel and Saimaa breeds) was extracted following the same protocol. Approximately 13 g of raw wool was placed in the thimble and extracted with 300 mL ethanol for 8 cycles (~4 h), with the cycle duration of 25–30 min. In all cases, extraction was complete when siphoning solvent back into the flask was visually colourless. Heating was achieved using heating mantles. To start the heating process, heat power was first set at 100% then reduced to 50% throughout the extraction. All experiments were conducted in triplicate. Once the extracts had cooled to room temperature, 5 mL samples were collected from each crude extract and stored separately for further UV-Vis analysis. The Soxhlet extraction process and representative extracts are presented in **Figure 7**.

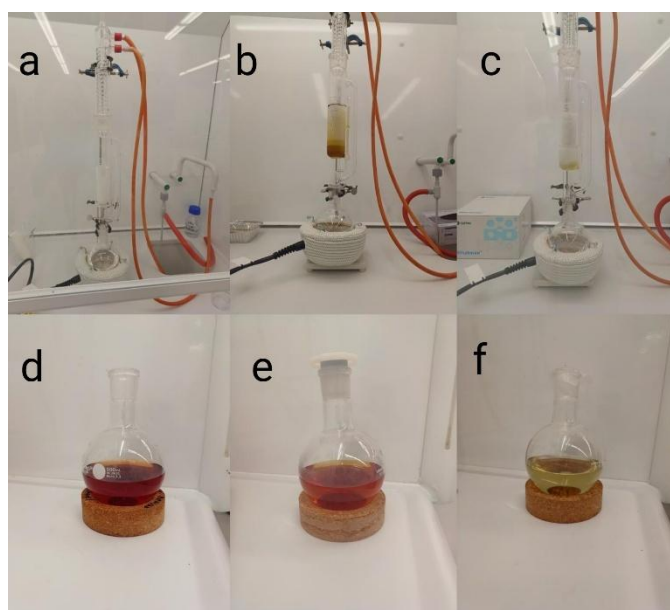


Figure 7. Soxhlet extraction process and obtained extracts. (a) Soxhlet apparatus setup before extraction, (b) during extraction with visible solvent circulation, (c) during last extraction cycle, (d) birch bark extract, (e) pine bark extract, and (f) Texel sheep wool extract.

3.3.3 Solvent immersion of birch bark

Birch bark was the only sample to be solvent immersed based on Bergvall *et al.*'s [52] procedure modified. Birch bark that was ground to <1mm and <4 mm particle size was used in the ratio of 1 g biomass to 20 mL ethanol (ETAX A), as was the biomass-solvent ratio used in Soxhlet extractions. The samples were placed in red-cap specimen containers and topped with aluminium foil to prevent light exposure and incubated at ambient temperature (25 °C) for 7 days with occasional manual stirring. All experiments were conducted in triplicate.

3.4. Post-extraction processing of different extracts

All the UAE, Soxhlet, and solvent immersion extracts were subjected to standard post-extraction procedures to ensure their clarity and stability before any additional analyses. The procedures included the removal of the solid residues by vacuum filtration, transferring the clarified extracts to suitable containers, and storage under temperature of -20 °C.

3.4.1 Vacuum filtration of cruciferous biomass and birch bark extracts

Vacuum filtration was applied on crude cruciferous biomass extracts and solvent-extracted birch bark extracts to remove insoluble impurities. The process was carried out using vacuum filtration setup equipped with a Büchner funnel and glass microfiber filter paper (GF/A, 70 mm diameter, 1.6 µm pore size; Whatman, China) was utilized for the filtration. Filtered filtrates were collected in sterile glass containers and reserved for additional processing and storage. The vacuum filtration setup is presented in **Figure 8**.

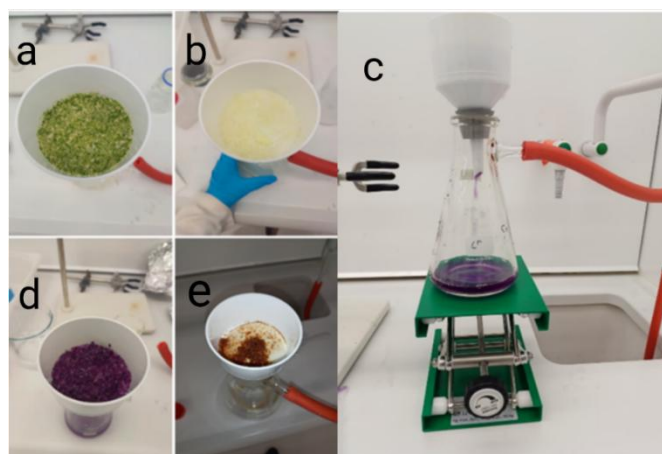


Figure 8. Vacuum filtration of different biomass extracts: (a) filtered broccoli, (b) filtered white cabbage, (c) filtration setup with Buchner funnel (d) filtered red cabbage, and (e) filtered birch bark extract.

3.4.2 Drying or concentration of different extracts

A portion of red cabbage and broccoli extracts was air-dried at room temperature in a fume hood for 2 days to obtain residues to be used in subsequent FTIR analysis. Some fractions of crude extracts of sheep wool and wood biomass were also air-dried under the same conditions for FTIR measurements. Furthermore, crude extracts of sheep wool and wood biomass were concentrated in a rotary evaporator (Heidolph Instruments GmbH, Germany) under low pressure (170 mbar) and 280 rpm, and at 40 °C, until the extract volume was reduced to approximately 40 mL. **Figure 9** illustrates the post-extraction processing of biomass extracts, including drying and rotary evaporation.

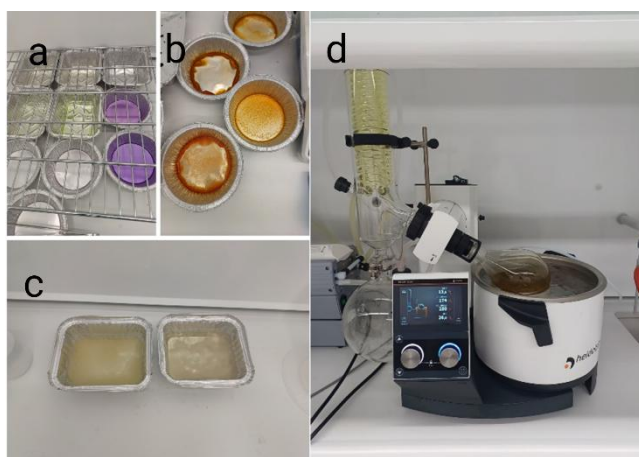


Figure 9. Drying and concentration of extracts: (a) drying of cruciferous biomass extracts, (b) drying of birch and pine bark extracts, (c) drying of sheep wool extracts, and (d) rotary evaporation of birch bark extract.

3.4.3 Storage conditions of extracts

All the extracts were stored in controlled conditions until further analysis. Liquid ethanol and aqueous extracts of crucifer biomass were stored at -20 °C. Air-dried residues intended for FTIR analysis were stored at room temperature in light and moisture-proof vials. Concentrated extracts made via rotary evaporation and 5 mL aliquots to be used for analyses in the future were kept at -20 °C.

3.5 Characterisation of extracts

Extracts from cruciferous biomass, wood bark, and sheep wool were characterized using an array of analytical techniques to determine their chemical structure and functional characteristics. Techniques employed for characterizing the extracts are as follows.

3.5.1 UV–Visible spectroscopy

The absorbance spectra of extracts were recorded on a UV–Visible spectrophotometer (Thermo Electron Corporation, UK) to provide preliminary information on molecules that absorb light, e.g., phenolics and other secondary metabolites. Samples were appropriately diluted in their own solvents (distilled water or ethanol) to be within the instrumental linear range of detection. Spectra were scanned in a wavelength range of 190–800 nm, and solvent blanks were used for baseline correction. All determinations were performed in triplicate.

3.5.2 Total phenolic content assay

Total phenolics were analysed by the colorimetric Folin–Ciocalteu method [96], using gallic acid as the standard. To each determination, 0.5 mL of appropriately diluted sample (or standard), 2.5 mL of a 10% (v/v) Folin–Ciocalteu reagent diluted with water, and 2.0 mL of Na₂CO₃ (7.5% w/v) were combined, shaken (10–15 s), and incubated in the dark at room temperature for 2 h. Absorbance was read at 760 nm on a UV–Vis spectrophotometer (Thermo Electron Corporation, UK). Triplicate readings were taken.

Calibration conditions

A 500 µg/mL stock of gallic acid was prepared (12.5 mg in 25 mL DI water). A six-point calibration series by serial dilution was built: 0, 50, 100, 200, 400, and 500 µg/mL. Water was used as the blank. Absorbance at 760 nm vs concentration was plotted and a linear regression (**Figure S 1**) was fit:

$$Y = 0.009 X + 0.0861 \quad (\text{Eq1})$$

where Y is absorbance and X is gallic acid concentration (µg/mL). The calibration curve demonstrated excellent linearity over the tested concentration range, with R²= 0.9943. Regression was used to determine the gallic acid equivalent concentration (C₁) of the

samples. Total phenolic content (TPC) was then expressed as gallic acid equivalents (GAE) per gram of extract using the equation:

$$TPC \left(\frac{mg \text{ GAE}}{g} \right) = \frac{C_1 \times V}{m} \quad (\text{Eq2})$$

where C_1 is the concentration of gallic acid equivalent (mg/mL) derived from the calibration curve, V is the extract volume (mL), and m is the extract mass (g).

3.5.3 High-performance liquid chromatography

Analysis of lipid classes was done on dried sheep wool extracts (Finnsheep and Texel breeds), commercial lanolin, Trico® repellent, and sheep fat. Lipids were first extracted using the Folch method and then determined by high-performance thin-layer chromatography (HPTLC) on CAMAG equipment.

The samples were analysed together on the same HPTLC plate. Lipid classes were separated and detected by the standard HiLIPID protocol of Helsinki University Lipidomics Unit (HiLIPID, Helsinki, Finland), where the analysis was carried out. The procedure offered a clear resolution and comparison of major lipid classes in the samples.

3.5.4 Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to determine functional groups in the extracts. The spectra were measured on a PerkinElmer Frontier spectrometer equipped with a diamond ATR (attenuated total reflectance) crystal between 400–4000 cm^{-1} . Resolution was 4 cm^{-1} , and three scans were averaged per spectrum. Spectra were corrected for ATR, baseline, and normalised prior to analysis.

3.5.5 Gas chromatography–mass spectrometry

Wood lipophilic extractives and pine bark were extracted by methyl tert-butyl ether (MTBE) and, following silylation, analysed by GC–MS. The analyses were conducted in Lappeenranta, campus of LUT University, by Dr. Liisa Puro with her established method [97].

3.6 Statistical analysis

Statistical calculation of the total phenolic content (TPC) data was performed using the R programming language (R Core Team, version 4.5.1). Results were expressed as mean \pm standard deviation (SD) of triplicate measurements. The experimental design included biomass (broccoli, cabbage, red cabbage), extraction method (UAE, freeze + UAE), solvent (DI water, ethanol), and temperature (25 °C, 50 °C) as independent variables. Due to missing combinations, the dataset was divided into three balanced factorial subsets to ensure valid testing of interaction effects.

Data processing involved defining factors consistently, followed by descriptive statistics and assumption checks for normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test). Extreme outliers were screened with *rstatix*. Because the full dataset was unbalanced and deviated from normality, non-parametric Aligned Rank Transform ANOVA (ART ANOVA, ARTool) was applied to the balanced models, with partial η^2 effect sizes calculated and classified by magnitude. Post-hoc comparisons were carried out using Tukey-adjusted emmeans tests, complemented by Cliff’s Delta effect sizes. Graphical visualisations, including interaction plots and summary graphs, were generated in OriginPro 2025 (OriginLab Corporation, Northampton, MA, USA; Copyrighted 1991–2025 OriginLab Corporation). Figure 10 presents a flowchart summarizing the overall research and analysis methodology applied in this study.

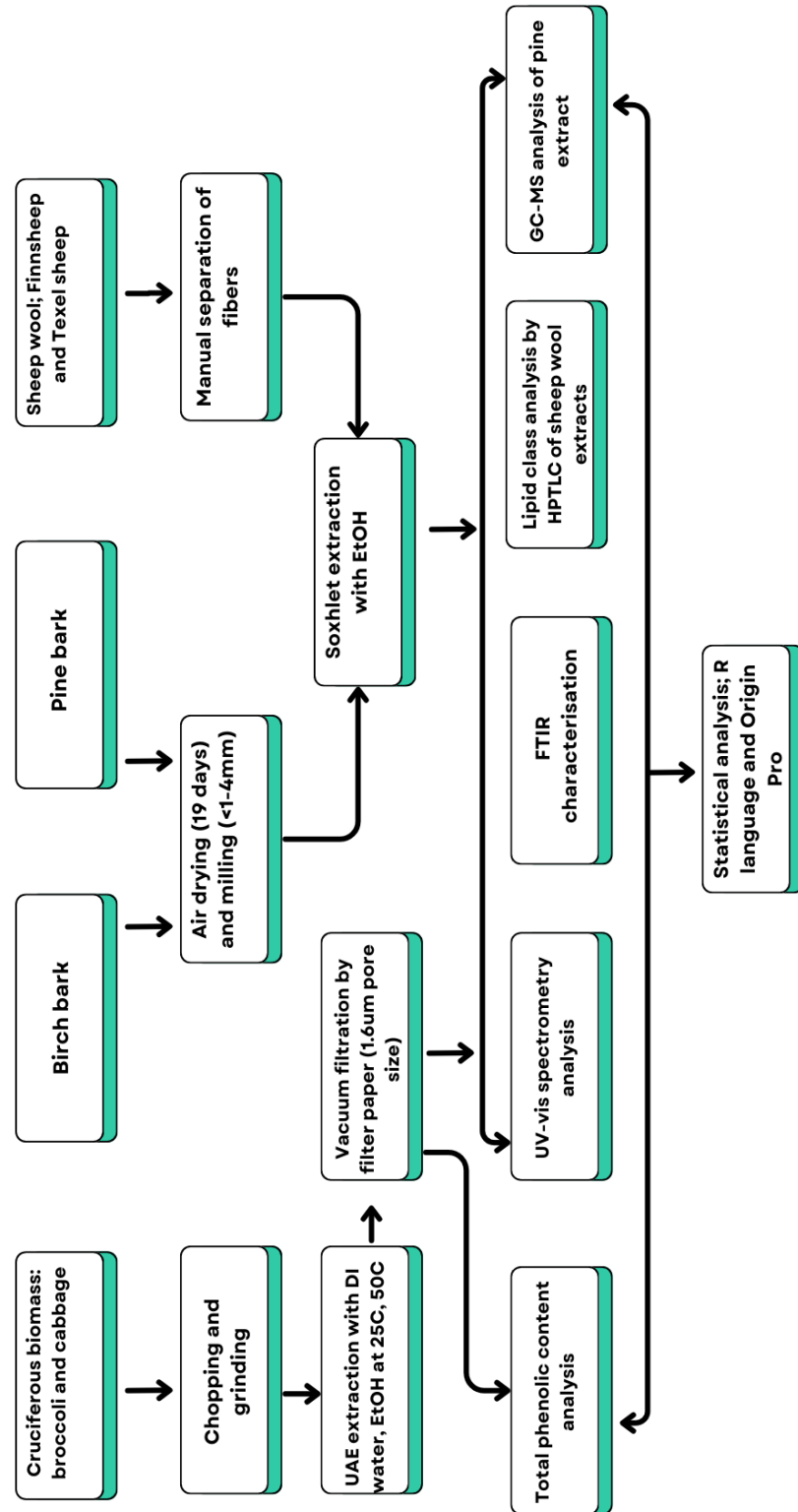


Figure 10. Flowchart of research and analysis methodology.

4. Results and discussion

This chapter presents the experimental result from the extraction and characterisation of different biomass sources. The focus is on their potential as natural repellents against browsing animals based on chemical composition and presence of bioactive compounds. The findings are presented by biomass type beginning with cruciferous vegetables, bark materials, and sheep wool. For every biomass, the findings are compared to literature values from published reports to highlight similarities, differences, and possible relevance to repellent formulation.

4.1 Characterisation of cruciferous biomass

Cruciferous vegetables including broccoli, cabbage, and red cabbage were investigated as prospective plant sources for repellent formulation. Presented herein are the experimental results of their extraction and characterisation. Qualitative findings by odour, colour, and effect of freeze pretreatment upon ultrasound-assisted extraction are the first descriptions presented. This is followed by spectroscopic and quantitative analysis including UV-Vis spectroscopy, total phenolic content, and Fourier-transform infrared spectroscopy (FTIR). Taken together, the foregoing findings give an overview of chemical composition and repellent-related characteristics of cruciferous biomass extracts.

4.1.1 Observations during ultrasound-assisted extraction of cruciferous biomass

Preliminary visual and sensory observations were made for the extracts of broccoli, cabbage, and red cabbage under different extraction conditions. Visual analysis of the effect of extraction method (UAE vs freezing (-20°C) + UAE), solvent (distilled water vs ethanol), and extraction temperature (25°C vs 50°C) on colour and odour of the extracts was conducted. Visual analysis was restricted to colour, turbidity, viscosity, and quality or characteristic smell. These qualitative characteristics can provide a general indication of pigment content, phenolic content, and possible development of volatile sulphur compounds typical of cruciferous vegetables. Summaries of the observations noted for all biomasses are presented in **Tables 5–7**.

Table 5. Visual and sensory observations of broccoli extracts by ultrasound-assisted extraction.

Method	Solvent	Temp (°C)	Colour description	Odor notes
UAE	Water	25	Pale green	Fresh vegetable, intense sulphurous aroma
UAE	Water	50	Bright green	Cooked vegetable, intense sulphurous aroma
UAE	EtOH	25	Light yellow green	Mild ethanol smell, less sulphurous aroma
UAE	EtOH	50	Yellow green	Strong ethanol, no noticeable aroma
Freezing+UAE	Water	25	Bright green	Fresh vegetable, robust fresh aroma with mild sulphur character
Freezing+UAE	Water	50	Dark green	Cooked vegetable, intense sulphurous aroma

Table 6. Visual and sensory observations of cabbage extracts by ultrasound-assisted extraction.

Method	Solvent	Temp (°C)	Colour description	Odor notes
UAE	Water	25	Light green	Fresh cabbage-like, with light sulphur notes
UAE	Water	50	Bright yellow green	Cooked cabbage, very light sulphurous aroma
UAE	EtOH	25	Pale yellow green	Mild ethanol
UAE	EtOH	50	Yellow brown	Strong ethanol
Freezing +UAE	Water	25	Yellow green	Faint fresh-cabbage aroma
Freezing +UAE	Water	50	Dark yellow green	Cooked cabbage

Table 7. Visual and sensory observations of red cabbage extracts by ultrasound-assisted extraction.

Method	Solvent	Temp (°C)	Colour description	Odor notes
UAE	Water	25	Purple	Moderate fresh-vegetable aroma with sulphurous odour
UAE	Water	50	Purple	Cooked cabbage, and stronger scent
UAE	EtOH	25	Pinkish purple	Mild ethanol
UAE	EtOH	50	Pinkish purple	Strong ethanol
Freeze +UAE	Water	25	Dark purple	Fresh cabbage
Freeze +UAE	Water	50	Darker purple	Cooked cabbage

a. Odour characteristics of cruciferous biomass extracts during UAE

Broccoli extracts possessed the most intense sulphurous smell compared to cabbage and red cabbage extracts, particularly in water UAE at 50 °C, which is consistent with high glucosinolate content in broccoli and subsequent hydrolysis of glucosinolates by myrosinase to form volatile isothiocyanates and other sulphur metabolites [74,89]. Increased aroma intensity at elevated temperature may reflect increased enzymatic action and volatilization of sulphur-containing metabolites. In cabbage extracts, sulphurous notes were generally weak and often detectable only in water extracts at 25 °C, reflecting a lower overall glucosinolate content in cabbage and variations in composition compared with broccoli. Red cabbage extracts were typically weaker, balanced in odour, with sulphur notes second only to fresh-vegetable odour, perhaps due to matrix effects of high anthocyanin content or compositional variation in glucosinolate profiles [74]. Ethanol extracts of broccoli and cabbage showed decreased sulphurous intensity compared to aqueous extracts. This may be expected due to ethanol's inhibitory effect on myrosinase, as it has been used in the inactivation of the enzyme [98], and decreased hydrophilic solubility of volatiles in less polar solvents, as ethanol has a lower hydrogen-bonding capacity relative to water [99].

b. Colour characteristics of cruciferous biomass extracts during UAE

Water extractions from broccoli and cabbage yielded green or yellow–green colours, which are attributed to the solubility of chlorophylls and hydrophilic flavonoids in polar solvents [74]. In comparison, ethanol extractions yielded lighter yellow–green or yellow–green colours, which was likely to arise from preferential solubilisation of less polar pigments such as carotenoids and certain flavonoid aglycones [74,89]. In the case of red cabbage, aqueous extracts preserved deep purple to dark purple colour, suggesting high water solubility of anthocyanins. Lighter pink–purple shades of ethanol extracts are consistent with lower anthocyanin solubility in less polar solvents while temperature effects were also apparent: extractions at 50 °C tended to give deeper colours, which may be a sign of higher release of pigments, but in some green extracts could also be due to chlorophyll degradation to pheophytin [74,89].

c. Effect of freeze pretreatment on cruciferous biomass extracts during UAE

Freezing at -20°C + UAE water extractions in all biomasses generally gave deeper colours compared to UAE alone. This agrees with the role of freezing in ensuring maximum extraction efficiency by inducing cell wall disruption through ice crystal growth, thereby

facilitating increased solvent penetration to intracellular pigments and phenolics [89]. In broccoli, freezing did not reduce sulphurous aroma substantially, indicating that myrosinase activity was retained under the treatment conditions. This result agrees with previous research that freeze-drying and freezing preserve myrosinase activity unless a thermal inactivation step is incorporated [74]. For cabbage and red cabbage, odour profile upon freezing was similar to UAE without freezing, but visual intensity of the extracts increased, particularly for aqueous extractions under high temperature.

4.1.2 UV-Visible spectroscopy of cruciferous biomass extracts for sulforaphane and glucosinolates

a. Water-based extractions of cruciferous biomass

Figures 11–13 present the UV-Vis absorbance spectra of broccoli, cabbage, and red cabbage aqueous extracts at different extraction conditions. Figure 11 presents the spectra of broccoli extracts (dilution factor, DF = 100), while Figures 12 and 13 present the spectra of cabbage and red cabbage extracts, respectively (DF = 50).

In accordance with Martínez-Zamora *et al.* [89], two deep-UV regions were of particular interest: 196 nm – SFN maximum absorbance, corresponding to the isothiocyanate functional group, and 227 nm – characteristic absorbance of desulfoglucosinolates, e.g., glucoraphanin, the predominant SFN precursor in broccoli.

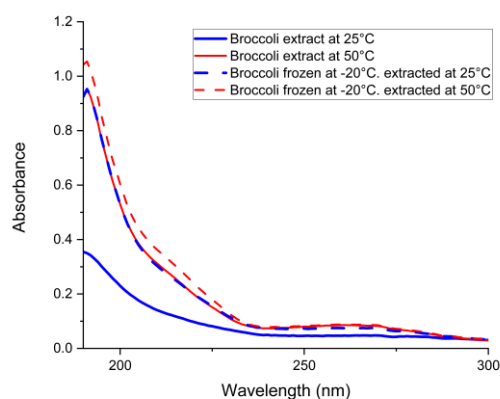


Figure 11. UV absorbance spectra of broccoli extracts obtained by ultrasound-assisted extraction under different conditions in distilled water: fresh samples extracted at 25 °C and 50 °C, (solid lines) and freeze-treated (–20 °C) samples extracted at 25 °C and 50 °C (dashed lines) with DF = 100.

Among all the tested cruciferous biomass extractions, broccoli extracts exhibited the highest absorbance in the region of 196–227 nm. Although this range may correlate to some extent with glucosinolates and their breakdown products, it should be taken into consideration that peaks in this region are generally broad and can be attributed to a series of UV-absorbing functional groups including conjugated carbonyl (C=O) groups, aromatic rings, amides, phenolic acids, flavonoids, terpenoids, and amino acids. Furthermore, high absorbance in the far-UV region (<200 nm) can also be induced by solvent effects (e.g., water) and other secondary metabolites like chlorophyll fragments and carotenoids [100–102], and the observed peak is therefore likely a composite response amplified by solvent effects.

Fresh cabbage extracted at 50°C revealed a definite increase in absorbance at 196 nm and 227 nm compared to fresh material extracted at 25 °C, which might suggest greater glucosinolate conversion and sulforaphane release with mild thermal treatment. The UAE at 50 °C with freeze pretreatment had the highest absorbance readings across the spectrum, confirming the hypothesis that cell wall disruption by ice crystals enhances compound release and mass transfer, as presented by Martínez-Zamora *et al.* [89]. Additionally, the increased spectral slope between 230 nm could also indicate the co-extraction of other UV-active secondary metabolites besides sulphur compounds.

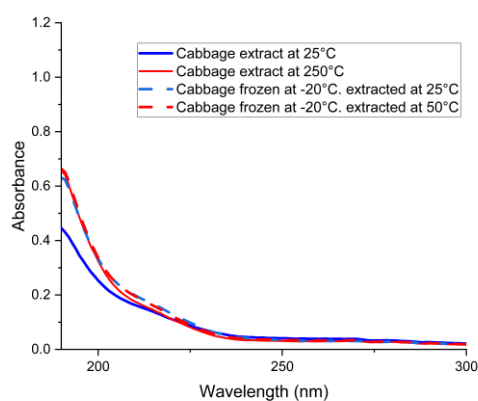


Figure 12. UV absorbance spectra of cabbage extracts obtained by ultrasound-assisted extraction under different conditions in distilled water: fresh samples extracted at 25 °C and 50 °C, (solid lines) and freeze-treated (–20 °C) samples extracted at 25 °C and 50 °C (dashed lines) with DF = 50.

The UV–Vis spectra for cabbage had detectable, though decreased intensity peaks at 196 nm and 227 nm compared to broccoli, which could be expected with lower total glucosinolate concentration and different glucosinolate composition. Interestingly, the thermal effect in cabbage was minimal; 50 °C extraction resulted in only a minor increase in

the absorbance at the SFN- and desulfoglucosinolate-related wavelengths of 196 and 227 nm, respectively, and the change in magnitude was relatively smaller compared to broccoli. Freeze pretreatment also caused a mild increase in absorbance at both extraction temperatures, though only marginally, which may suggest that the pool of glucosinolates in cabbage is not so sensitive to physical disturbance alone. The absorbance settled rapidly below 230 nm, which suggests fewer co-extracted conjugated or aromatic compounds of UVB range compared to broccoli.

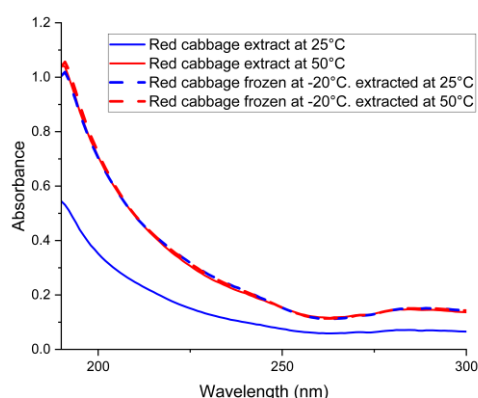


Figure 13. UV absorbance spectra of red cabbage extracts obtained by ultrasound-assisted extraction under different conditions in distilled water: fresh samples extracted at 25 °C and 50 °C, (solid lines) and freeze-treated (−20 °C) samples extracted at 25 °C and 50 °C (dashed lines) with DF = 50.

Red cabbage spectra were characterised by a pronounced absorbance rise in the deep UV region (190–210 nm), similar to cabbage and broccoli, but treatment-related differences were minimal. The highest intensities at 196 nm were observed in samples pretreated by freezing at both extraction temperatures, although the increase compared to fresh extractions was small. The influence of temperature was subtle, with 50 °C extractions producing slightly higher deep-UV absorbance in frozen samples but negligible differences in fresh samples. The limited variability in the SFN-relevant range may be attributed to the pigment composition of red cabbage, which is dominated by anthocyanins and other polyphenolic pigments that absorb primarily in the visible range (>500 nm). The stability of anthocyanin content in aqueous extractions may overshadow subtle differences in sulphur-compound release across treatments [103].

Comparison of the spectra across the three biomasses indicated that broccoli had the highest absorbance at both 196 nm and 227 nm, particularly when pretreatment by freezing and UAE were used in combination at 50 °C. This indicates a greater capacity for releasing SFN and associated sulphur-containing volatiles under optimised extraction conditions.

Cabbage showed intermediate absorbance values, whereas red cabbage showed the lowest variability towards treatments in the SFN-related region, perhaps due to its anthocyanin-rich but comparatively sulphur-moderate composition.

Earlier compositional studies validate these findings, with Martínez-Zamora *et al.* [89], having determined glucoraphanin levels of 5.50 ± 0.11 g kg⁻¹ DW in broccoli florets and 5.01 ± 0.07 g kg⁻¹ DW in broccoli leaves, corresponding to sulforaphane levels of 5.51 ± 0.07 g kg⁻¹ DW and 2.52 ± 0.09 g kg⁻¹ DW, respectively. These measurements verify the considerable SFN release capacity of broccoli compared to other cruciferous vegetables. On the contrary, cabbage has been shown to have lower total glucosinolate content and reduced levels of precursors of SFN, and although the red cabbage is rich in anthocyanins, it tends to contain less glucoraphanin than green broccoli types [69,84].

b. Ethanol-based extractions of cruciferous biomass

UV-Vis absorbance spectra of broccoli, cabbage, and red cabbage aqueous extracts obtained by UAE in EtOH at different extraction conditions are presented in **Figure 14**.

Ethanol extracts of broccoli had the highest absorbance at 196 nm and 227 nm among the extracts of cabbage and red cabbage, with increased signals at 50 °C compared to 25 °C, demonstrating that mild heat in ethanol might enhance SFN and glucosinolate release. Despite ethanol's ability to inhibit the activity of myrosinase, presence of these peaks may suggest some activity of the enzyme or direct extraction of more lipophilic SFN derivatives. Absorbance at moderate level at 270–300 nm shows co-extraction of ethanol-soluble aromatic compounds particularly phenolic acids, flavonoids, and other aromatics [104,105].

Cabbage extracts showed less absorbance that could be associated to SFN- and glucosinolate-associated absorbance, with minimal temperature dependency, except broad features > 230 nm possibly attributable to reduced glucosinolate content and reduced ethanol-soluble aromatics. Intermediate absorbance was found in red cabbage extracts, with minimal enhanced peak at 50 °C and more extensive absorbance 260–320 nm, likely a consequence of partial polyphenolic and anthocyanin extraction, although these contribute minimally to deep-UV peaks.

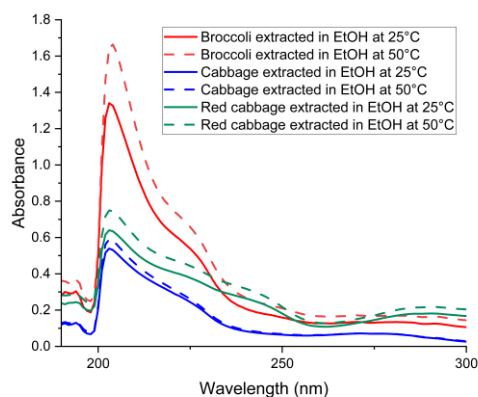


Figure 14. UV absorbance spectra of fresh samples of broccoli, cabbage, and red cabbage extracts obtained by ultrasound-assisted extraction in EtOH extracted at 25 °C (solid lines) and 50 °C (dashed lines) with DF = 15.

Overall, water-based extractions exhibited possibly higher SFN and glucosinolate-related absorbance at 196 nm and 227 nm for broccoli, cabbage, and red cabbage extracts. This is consistent with literature reporting aqueous media to be preserving and promoting for myrosinase activity, ensuring efficient hydrolysis of glucoraphanin into SFN and related isothiocyanates [74,89,106]. Water also provides selectivity in the instance of polar bioactive compounds, e.g., hydrophilic sulphur metabolites (e.g., glucosinolates, isothiocyanates), phenolic acids, flavonoid glycosides, and anthocyanins, with avoidance of co-extraction of lipophilic or unwanted non-polar compounds [107]. On the other hand, ethanol extracts consistently caused diminished absorbance in SFN- and glucosinolate-associated UV regions, possibly indicating partial myrosinase inhibition by ethanol and higher polarity-dependent lower solubility for glucosinolates. However, ethanol can extract formed SFN and certain sulphur metabolites, and minimal heating (50°C) increased extraction efficiency of UV active compounds in the present findings, particularly for broccoli and red cabbage.

Although UHPLC is the standard method of quantitation of sulforaphane (SFN) and its glucosinolate precursor glucoraphanin, the spectral characteristics reported in Martínez-Zamora *et al.* [89], are useful as benchmarks for interpretation of UV absorbance profiles in cruciferous extracts. Glucoraphanin, a desulfoglucosinolate, was 227 nm, and SFN was 196 nm, in their UHPLC on a Gemini C18 column. These wavelengths also correspond to strong absorbance of the thiourea and isothiocyanate functional groups, making them selective for sulphur-containing metabolites of glucosinolate hydrolysis. While UV–Vis spectra recorded in the present study were scanned over a broader range, the presence of peaks or shoulders

at around these wavelengths can be possibly used as indirect confirmation of SFN- or glucosinolate-containing compounds in the extracts.

Broccoli was identified as the most suitable raw material for browsing repellent development, exhibiting the strongest sulphurous odour and UV absorbance at wavelengths of 196 and 227 nm, consistent with potentially high sulforaphane and glucosinolate contents. Cabbage and red cabbage contained less intense sulphur profiles, and red cabbage was characterised by anthocyanins rather than sulphur metabolites. Water-based ultrasound-assisted extraction at 50°C, with pretreatment by freezing at -20°C, maximised the release of sulphur compounds. This condition maximised the release of glucosinolates and their hydrolysis product (SFN, isothiocyanates), which are responsible for the pungent sulphurous odour, and are expected to be key active deterrent compounds against browsing animals.

4.1.3 Total phenolic content analysis of cruciferous extracts

Figure 15 represents a heatmap representation of the average TPC (mg GAE/g DW) of red cabbage, cabbage, and broccoli under six different combinations of extraction conditions. Conditions are defined by the combination of extraction method, solvent, and extraction temperature. Values in each cell are the average TPC of three replicates, and a colour intensity is depicted by a gradient in which darker red colours correspond to higher TPC values. This presentation enables rapid identification of optimal conditions for phenolics' extraction in all biomass types.

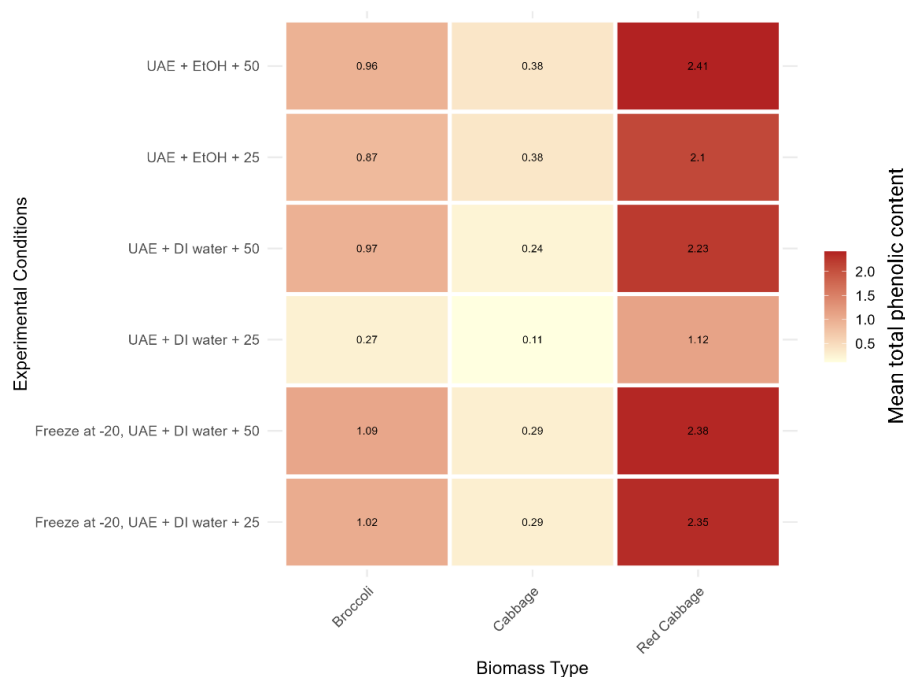


Figure 15. Heatmap of total phenolic content (mg GAE/g) in broccoli, cabbage, and red cabbage extracts under different extraction conditions. Rows represent extraction treatments, including ultrasound-assisted extraction with ethanol or deionized water at 25 °C or 50 °C, and freeze pretreatment (−20 °C) followed by ultrasound-assisted extraction. Columns represent biomass types. Colour intensity corresponds to TPC values, with darker red shades indicating higher phenolic content. Numerical values within cells show the mean TPC for each biomass–treatment combination.

Red cabbage registered the highest TPC values under all conditions studied, a reflection of its phenolic-rich phytochemical composition dominated by anthocyanins, flavonols, and hydroxycinnamic acids. Upon water extraction, raising temperature from 25 to 50 °C moderately increased TPC for broccoli and cabbage but had a more significant enhancing impact for red cabbage, particularly when combined with pretreatment by freezing. This aligns with cell disruption chemistry: freezing induces the formation of ice crystals, which cause cell walls and membranes to break, favouring the release of phenolics sequestered in vacuoles during UAE.

Even higher TPC values for red cabbage at 50 °C were achieved in ethanol extractions, in line with ethanol's intermediary polarity being better suited to solubilise anthocyanins in their flavylum cation form, and moderately polar flavonoids. Broccoli and cabbage contained lower TPC in ethanol compared to red cabbage, which indicated their lower content of ethanol-soluble phenolics and greater contents of hydrophilic compounds such as chlorogenic acid and certain hydroxycinnamates that are better extracted in water.

Table 8. presents the three-way ANOVA results for TPC values of cruciferous biomass extracts by UAE in terms of the main effects of biomass type, solvent, and extraction temperature. All two-way and three-way interaction effects between the factors

are also presented in the table in a comprehensive assessment of their individual and interactive impact on the TPC values.

Table 8. Three-way ANOVA results for total phenolic content, showing significant effects of biomass type, solvent, extraction temperature, and their interactions (***, $p < 0.001$), Degrees of freedom (df) reflect the number of levels minus one for each factor, and F-values indicate the ratio of explained to unexplained variance.

Factor	Df	Sum Sq	F value	p value	Significance	Partial Eta ²
Biomass	2	3456	98.04255	2.83×10^{-12}	***	0.8910
Solvent	1	2916	75.33262	7.24×10^{-9}	***	0.7584
Temperature	1	2916	75.03645	7.51×10^{-9}	***	0.7577
Biomass * Solvent	2	3407	88.68691	8.21×10^{-12}	***	0.8808
Biomass * Temperature	2	3462	101.39626	1.97×10^{-12}	***	0.8942
Solvent * Temperature	1	2916	74.8492	7.68×10^{-9}	***	0.7572
Biomass * Solvent * Temperature	2	3220	59.9731	4.61×10^{-10}	***	0.8333

Significance codes: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Three-way ANOVA results show that all the main factors had highly significant effects on total phenolic content (TPC) ($p < 0.001$ for all). Of these, the most influential was biomass type ($F = 98$), reflecting the inherent chemical diversity in phenolic content and composition between broccoli, cabbage, and red cabbage. The pre-eminence of biomass is an affirmation that genetic and structural factors, cell wall composition, vacuole pigment load, and the proportion of bound to soluble phenolics, are fundamental drivers of extraction yield.

Solvent was the next most prominent major factor ($F > 75$), which also confirmed the crucial role of solvent polarity in phenolic recovery. Ethanol selectively removed anthocyanins and more polar flavonoids, leading to higher TPC in red cabbage, while water extracted polar phenolic acids more efficiently in favour of broccoli and cabbage. Temperature ($F = 75$) greatly improved efficiency of extraction, perhaps by increasing solute solubility, reducing viscosity of the solvent, and increasing diffusion of phenolics into the solvent. Extraction method also had significant impacts on extraction ($F = 74$), with freeze pretreatment raising yields by bringing about ice crystal damage to cell walls, releasing phenolics stored in vacuoles (*Table S 1*)

The significance of two-way interactions implies that the impact of one factor depended on the levels of another. The solvent \times biomass interaction, for instance, illustrates that the choice of solvent was most critical for red cabbage due to its anthocyanin

composition, but less critical for cabbage. The interaction temperature \times method suggests phenolic yield from red cabbage was more enhanced by heat than from cabbage or broccoli. The method \times temperature interaction suggests that pretreatment by freezing was particularly effective at higher extraction temperatures, likely due to a synergistic effect between physical cell breakage and thermally improved solubilisation. On the other hand, highly significant three-way interactions—biomass \times method \times temperature and biomass \times solvent \times temperature; highlight maximum extraction strategies being biomass-specific. Ethanol and elevated temperature and pretreatment by freezing, for example, were optimal for red cabbage, while broccoli was optimally extracted using water at 50 °C with pretreatment by freezing (*Table S 1* and *Table S 2*).

Figure 16 depicts the average total phenolic content (mg GAE/g) of the three biomass types tested: broccoli, cabbage, and red cabbage. Each bar indicates the mean of three independent replicates, and error bars represent the standard error of the mean. Different small letters above the bars indicate statistically significant differences between biomass types, as determined by Tukey's HSD post-hoc test following three-way ANOVA ($p < 0.05$).

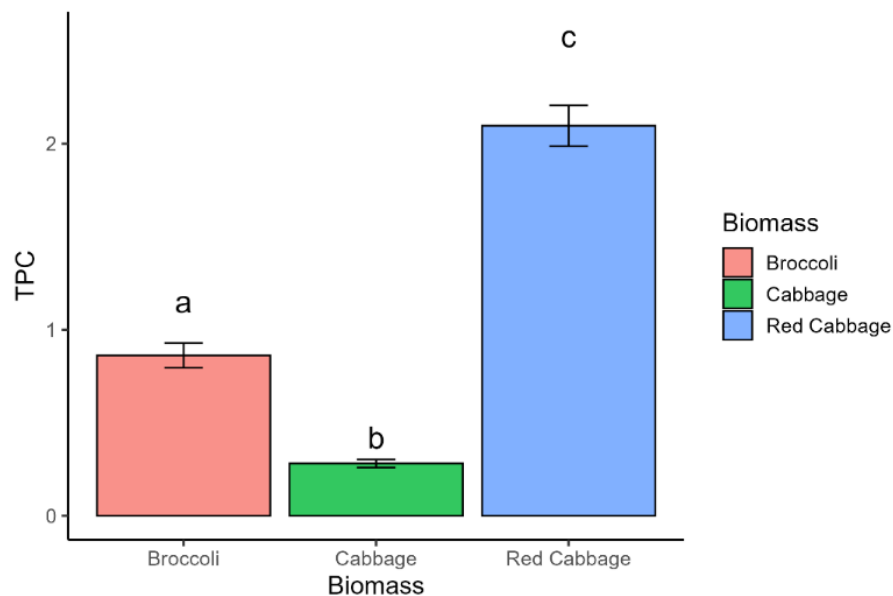


Figure 16. Effect of biomass type on total phenolic content (TPC, mg GAE/g DW). Bar plots represent mean TPC values \pm standard error (SE) for broccoli, cabbage, and red cabbage extracts across treatments. Different letters (a–c) above the bars indicate statistically significant differences ($p < 0.05$) between biomass types according to Tukey's post-hoc test.

Post-hoc Tukey HSD tests confirm that TPC differed significantly between all three biomass types ($p < 0.001$ for each pairwise comparison). Red cabbage and cabbage were significantly different, with red cabbage containing over seven times the phenolic content of

cabbage under the tested extraction conditions. Red cabbage contained considerably higher levels of phenolic compounds compared to broccoli. While the difference in phenolic content between broccoli and cabbage was smaller, it remained statistically significant, with broccoli still clearly exceeding cabbage in phenolic content.

Trends have been equally observed in previous research; Zafar *et al.* [108], quantified TPC in five Brassica species which were isolated under a shared Soxhlet–ethanol condition. They found that red cabbage (32.8 mg GAE/g DW) had significantly higher TPC value than broccoli (27.2 mg GAE/g DW) and white cabbage (16.4 mg GAE/g DW) ($p < 0.05$), the same ranking and statistical difference identified in our Tukey HSD comparisons. Similarly, Parada *et al.* [108], also accounted for considerably higher phenolic content in red cabbage compared to Chinese and white cabbage, citing literature values for red cabbage as 1851 mg GAE/100 g DW compared to white cabbage at 980–1220 mg GAE/100 g DW. These duplicated multi-study trends for extraction methods and regions of origin substantiate the conclusion that red cabbage is inherently more phenolic than white cabbage or broccoli, and broccoli always performs better than white cabbage.

The trends of TPC obtained in this study have some points in common with the findings of Martínez-Zamora *et al.* [89], who studied phenolic recovery of freeze-dried broccoli by-products (leaves and florets) using UAE in water with different solid-to-liquid ratios (2:25 and 1:25 g/mL), extraction temperatures, and times. In their work, TPC increased with temperature up to 55 °C, with peak values observed at the ratio of 1:25. Lower recovery of phenols at 2:25 was accounted for by lower availability of solvent per unit weight, decreasing solubilisation and diffusion of phenolic compounds despite identical extraction conditions. Ratio effect was particularly noticeable in the case of broccoli leaves that contain a higher proportion of cell wall-bound phenolics that require efficient penetration by solvent to release.

In comparison, the present study was conducted at a fixed ratio of 2:25 for all three of the biomasses, with a lowered extraction temperature range (25 °C and 50 °C) and extraction time fixed at 20 min. Under these conditions, TPC values for cabbage and broccoli were lower than the maximum values reported by Martínez-Zamora *et al.* [89] for 1:25 ratio because larger volumes of the solvent enhance the efficiency of phenolic extraction. The solvent limitation at 2:25 is likely more important in fresh material due to it having higher

water content, likely reducing effective solvent-to-solid contact compared to freeze-dried material.

Although red cabbage exhibited the highest total phenolic content, its phytochemical composition is marked by anthocyanins, which contribute mainly to colour, antioxidant potential, and mild astringency, but lack the volatile sulphurous character required for browsing deterrence. In contrast, broccoli had possibly much higher levels of glucosinolates and sulforaphane, which hydrolyse to volatile isothiocyanates that provide the strong pungent aroma responsible for repellent efficacy against deer and moose. Thus, broccoli remains the optimal biomass for repellent development, with red cabbage serving as a support that could be employed for stabilising the extracts through antioxidant protection or persistence by UV absorbance, but not as the principal source of active deterrent compounds.

4.1.4. Fourier-transform infrared spectroscopy (FTIR) to identify characteristic functional groups in cruciferous biomass extracts

To further characterise the chemical composition of cruciferous biomass extracts, Fourier-transform infrared spectroscopy was applied to broccoli and red cabbage extracts obtained by UAE in water and EtOH. FTIR analysis provides information on functional groups of compounds present in cruciferous vegetables extracts, including proteins, carbohydrates, phenolics, and sulphur-containing compounds. Figures 17 and 18 show the FTIR spectra water and EtOH extracts of cruciferous biomass obtained from different extraction conditions.

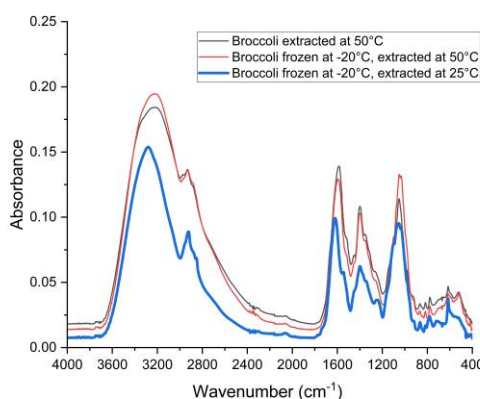


Figure 17. FTIR spectra of water extracts of broccoli obtained from different extraction conditions. Spectra compare broccoli extracted at 50 °C, frozen at -20 °C and extracted at 50 °C, and frozen at -20 °C and extracted at 25 °C by ultrasound-assisted extraction.

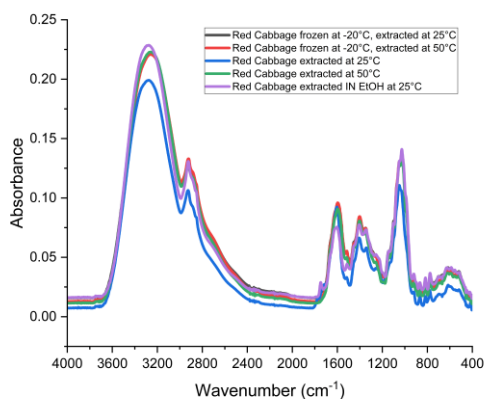


Figure 18. FTIR spectra of water extracts of red cabbage obtained from different extraction conditions. Treatments include extractions at 25 °C, 50 °C, and and freezing pretreatment combined (at -20°C) combined with ultrasound-assisted extraction in water, as well as extraction at 25 °C with ethanol.

The FTIR spectra of broccoli and red cabbage extracts obtained by UAE showed characteristic bands corresponding to major phytochemical groups of carbohydrates, phenolics, and sulphur-containing compounds. Both extracts exhibited a broad and intense band in the $\sim 3300\text{ cm}^{-1}$ region, attributed to O–H and N–H stretching vibrations, indicating the presence of hydroxyl-rich polysaccharides, proteinaceous components, residual water, and alcohols [109,110]. Freezing pretreatment prior to ultrasound-assisted extraction of broccoli and red cabbage extracts results in a stronger broad absorption in the $\sim 3300\text{ cm}^{-1}$ region compared with non-pretreated extracts. This enhancement is attributed to freeze–thaw–induced cell breakage, which enables the release of intracellular polysaccharides and proteins, thereby enhancing the occurrence of hydroxyl and amine groups in the extracts [111]. However, it is important to note the slight difference in the $\sim 3300\text{ cm}^{-1}$ region between red cabbage and broccoli extracts, which can be explained by higher concentration of anthocyanins and other polyphenolic compounds in red cabbage [112], which contain abundant hydroxyl groups capable of forming extensive hydrogen bonding networks.

Peaks around 2920 cm^{-1} and 2850 cm^{-1} are representative of asymmetric and symmetric C–H stretching vibrations of aliphatic chains, which are common in lipids and fatty acids, proteins, and carbohydrates [109,110]. While the $1500\text{--}1700\text{ cm}^{-1}$ spectral region is mainly associated with vibrations of amide I and II [110]. Of particular interest are $\sim 1635\text{ cm}^{-1}$ and $\sim 1646\text{--}1652\text{ cm}^{-1}$ bands representing N–H deformation and C=O stretching vibrations of amide I groups, respectively [110], as well as aromatic ring stretching and strong C=C peaks of alkenes [113], with clear differences in absorbance intensity between red cabbage and broccoli extracts. Additional peaks at 1099 and 1241 cm^{-1} are attributed to

symmetrical and asymmetric stretching of ester C–O–C bonds, while the bands near 1733 and 1749 cm^{-1} are attributed to ester carbonyl stretches [110].

Glucosinolates have a unique C=N–sulphate moiety responsible for strong IR absorptions between 1630–1690 cm^{-1} superimposed over amide I bands [110,114]. Their β -D-thioglucose compound also has distinctive absorptions in the range 1000–1200 cm^{-1} (C–O stretching) [115]. Variations in the profiles of extracts have been noted among aliphatic glucosinolates with open hydrocarbon chains and closed indole ring glucosinolates; the latter usually introduce extra vibrations in the 1350–1650 cm^{-1} region [110,113] which among their hydrolysis products, sulforaphane and indole-3-carbinol are especially noteworthy, being derived from glucoraphanin and glucobrassicin, respectively [116].

In red cabbage extracts, the intensified band at \sim 1515–1600 cm^{-1} indicates the presence of anthocyanins and flavonoids [113] as it corresponds to the C=C skeletal stretching vibrations of their aromatic rings resulting in stronger IR absorbance compared to non-aromatic molecules [117], whereas broccoli spectra are more dominated by glucosinolate-associated peaks in the sulphate ester region of 1350–1650 cm^{-1} . Solvent-dependent behaviours were noticed as well. Extractions with water favoured hydrophilic compounds such as glucosinolates and carbohydrates, while phenolic C=C stretching is typically observed as a smaller band near 1580 cm^{-1} [109], extractions with ethanol showed stronger absorbance closer to \sim 1650 cm^{-1} , more consistent with amide I vibrations and carbonyl stretching [110] rather than just phenolic groups. Temperature also played a role, with extractions done at 50 °C following the freeze pretreatment generally reporting stronger absorbance in both broccoli and red cabbage extracts, indicating increased solubilization of phytochemicals under mild warming.

These findings confirm earlier assumptions that broccoli extracts by UAE in water contain higher content of sulphur-glucosinolates than red cabbage, which is consistent with the intense sulphate ester and isothiocyanate-related FTIR peaks presented in Figure 15. This sulphur-rich phytochemical profile further supports the evidence for broccoli being a more promising candidate for repellent formulation.

4.2. Characterisation of bark biomass

Birch and pine bark were characterised to determine their structural features relevant to repellent formulation, particular attention was given to their moisture content and extractive-rich fractions, which are known to vary between species.

4.2.1. Moisture content analysis in bark samples

Table 9 presents the moisture content of birch and pine bark before and after air-drying at room temperature for 19 days

Table 9. Moisture content (%) of birch and pine bark before and after air-drying at room temperature for 19 days.

Biomass type	Moisture content %	
	Pre-drying	Post-drying
Birch bark	6.36	4.17
Pine bark	66.02	9.09

Moisture content plays an important role in the effectiveness of solvent-based extraction from bark biomass. Birch bark, which naturally contains a low water content, decreased from 6.36% to 4.17% after drying, which may lead to a minimal influence on the extraction efficiency. Birch outer bark is naturally hydrophobic and rich in pentacyclic triterpenoids such as betulin (10–40% dry weight), which are efficiently extracted after air-drying the biomass [77]. Different studies highlight that pre-drying is routinely performed to achieve maximum solvent penetration in the absence of water interference, although over-drying may lead to the degradation of further heat-sensitive molecules including phenolic acids (e.g., protocatechuic acid, *p*-coumaric acid, ferulic acid), glycosylated flavonoids are especially prone to hydrolysis and degradation with heat, and volatile terpenoids [52,77].

In contrast, pine bark is highly susceptible to moisture, and typically contain 50–70% water, as observed here at 66.02%, which decreased to 9.09% after drying. Drying is a crucial step because excess water inhibits diffusion of solvents into resin ducts, dilutes target compounds, and encourages co-extraction of sugars and polar impurities [75]. Mostly after drying, Soxhlet and similar extraction methods recover lipophilic components, such as resin acids (abietic, dehydroabietic, palustric acids at 20–60 mg/g) and monoterpenes (α -pinene and 3-carene at 22–91 mg/g) in yields comparable to those reported in the literature [56,118].

Thus, while birch bark may be successfully extracted with minimal drying, pine bark must undergo substantial moisture reduction to achieve reproducible and effective recovery of its bioactive fractions.

4.2.2 UV-Visible spectroscopy of birch extracts

UV-Vis spectra of ethanol extracts of birch bark demonstrates significant differences in the absorbance intensity depending on both the extraction method (Soxhlet vs. solvent immersion) and particle size of bark material as shown in Figure 19. Soxhlet extraction of 4 mm bark yielded the maximum values of absorbance, particularly in the region of UV 200–230 nm, where strong absorption indicates conjugate systems, such as phenolic acids and triterpenoids [52]. In contrast, solvent immersion at room temperature resulted in lower absorbance, as the lack of heating limits solvent penetration and solubilisation of hydrophobic compounds [77]. The difference in particle size also contributed to the extraction effectiveness: smaller particles (1 mm) provided higher surface area for interaction with the solvent, resulting in slightly higher absorbance compared to 4 mm particle size.

Betulin, the most common triterpenoid in birch bark, is typically determined by UV absorbance at 200–220 nm, sometimes extending up to 260–280 nm [1]. The large peak in this lower part of the UV spectrum in Figure 21 aligns with the presence of betulin, along with other ethanol-soluble extractives. The stronger absorbance detected in the Soxhlet extract suggest that continuous reflux of the solvent at elevated temperatures not only improves diffusion into the bark matrix but also promotes the recovery of poorly soluble triterpenoids, such as botulin [52,77]. Generally, these results suggest that thermal energy and particle size reduction influence the efficiency of extraction, with Soxhlet extraction offering a clear advantage over room-temperature immersion for the recovery of bioactive compounds from birch bark.

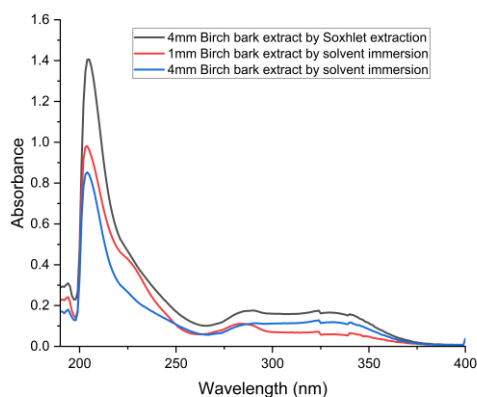


Figure 19. UV-Vis spectra of birch bark extracts in ethanol obtained by Soxhlet extraction (4 mm, black) and solvent immersion (1 mm, red; 4 mm, blue) at $DF = 300$.

4.2.3 Fourier-transform infrared spectroscopy (FTIR) of bark extracts

Fourier-transform infrared (FTIR) spectroscopy was performed to identify the main functional groups present in the ethanol extracts of pine and birch bark by Soxhlet extraction. The resulting spectra (Figures 20 and 21) highlight characteristic absorption bands associated with phenolic compounds, triterpenoids, resin acids, and other bioactive constituents relevant to repellent activity.

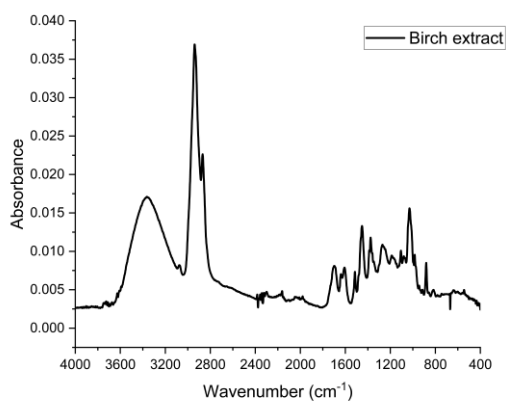


Figure 20. Infrared spectrum of ethanol extract of birch bark obtained by Soxhlet extraction.

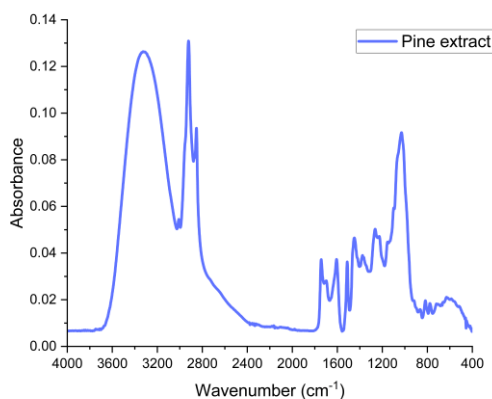


Figure 21. Infrared spectrum of ethanol extract of pine bark obtained by Soxhlet extraction.

The FTIR spectra of birch and Scots pine bark obtained by Soxhlet extraction in EtOH (Figures 18 and 19, respectively) showed a broad absorption at 3600–3200 cm^{-1} , resulting from O–H stretching vibrations of alcohols', phenolics', and carboxylic acids' hydroxyl groups [113,114]. This band was more intense in the pine extract, which indicated a higher content of hydroxyl-rich resin acids and polyphenols compared to birch, consistent with previous study by Routa *et al.* [50]. Distinct aliphatic C–H stretching vibrations were shown in the 2853–2924 cm^{-1} region for both extracts, which is attributed to methylene ($-\text{CH}_2$) and methyl ($-\text{CH}_3$) groups of long aliphatic chains typical of fatty acids, triglycerides and sterols [114]. Such bands were considerably more intense in pine extract due to its rich content of lipophilic resin and fatty acids [55].

Differences between pine and birch extracts were observed in the carbonyl stretching region. Birch extract displayed a sharp peak around 1710 cm^{-1} , characteristic of carboxylic acid C=O stretching, and consistent with the occurrence of betulinic acid and other triterpenes [119]. In contrast, pine extract showed a more intense band at around 1730 cm^{-1} for ester carbonyl groups in triglycerides, steryl esters, and resin acid esters [113,114], due to the prevalence of esterified lipids in pine bark. Peaks in the 1510–1600 cm^{-1} region were attributed to lignin-derived structures with aromatic C=C skeletal vibrations for both extracts [120,121]. There were also peaks at 1030–1245 cm^{-1} owing to the C–O stretching of ethers, esters, and phenolic groups, as well as secondary alcohols and residual polysaccharides [109,121]. A very typical peak in the birch extract spectrum was the one at approximately 887 cm^{-1} , attributed to the =C–H out-of-plane bending of exomethylene groups [122] Which is diagnostic band for pentacyclic triterpenes such as betulin, the main component of birch

bark extract [53,123]. Such a band was not found in the pine extract, again confirming the species-specific differences in extract composition.

Overall, FTIR analysis demonstrated some compositional differences between birch and pine extracts. Birch bark was characterized by the presence of triterpene and carboxylic acid (betulin, betulinic acid) bands, as well as rich lignin contributions. Whereas pine extract contained stronger ester carbonyl signals, abundant aliphatic chains, and lignin markers, due to its higher content of resin acids, steryl esters, and fatty acids.

4.2.4 Gas chromatography–mass spectrometry analysis of lipophilic composition of pine extracts

The lipophilic composition of pine extracts was analysed by GC-MS. Major compound groups measured included lignin residuals, fatty acids, resin acids, lignans, sterols, steryl esters, triglycerides, and total lipophilics. Their mean concentrations are shown in Figure 22.

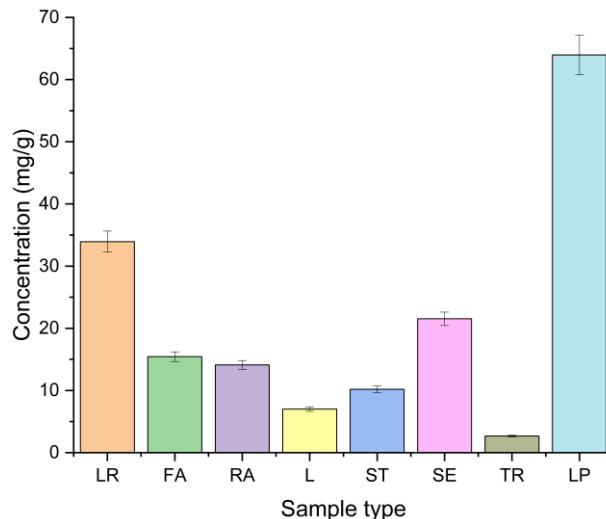


Figure 22. Mean concentrations of lipophilic compounds in pine extracts determined by Gas chromatography–mass spectrometry (mg/g). Error bars correspond to 5% of the mean values, calculated in OriginPro. Abbreviations: LR = lignin residuals; FA = fatty acids; RA = resin acids; L = lignans; ST = sterols; SE = steryl esters; TR = triglycerides; LP = lipophilics.

GC–MS analysis of pine bark extracts in EtOH obtained by Soxhlet extraction showed that lipophilics and lignin residuals were the dominant extractive fractions (≈ 64 mg/g and 34 mg/g, respectively). Lipophilic extractives are further divided into fatty acids, resin acids, sterols, steryl esters and triglycerides, out of which fatty acids (FA ≈ 12 mg/g), sterols (≈ 7 mg/g), and resin acids (≈ 5 mg/g) were more abundant. The fractions of lignans

(≈ 3 mg/g), steryl esters (≈ 2 mg/g), and triglycerides (≈ 1 mg/g) were minor. This composition highlights the dominance of esterified sterols and lignin-derived residues, while fatty and resin acids contribute substantially to the volatile and bioactive profile. Compared with published data [75,123], the observed concentrations align with reported ranges but vary slightly depending on the source of Scots pine and extraction method.

Anäs *et al.* [123], reported that outer pine bark (*Pinus sylvestris*) contained 9 mg/g of fatty acids while inner pine bark contained ≈ 38 mg/g, primarily linoleic, oleic, and pinolenic acids. They also found higher concentrations of resin acids in inner pine bark compared to outer pine bark (7mg/g and 2 mg/g, respectively), mostly in the form of abietic acid compounds. In another study, Zommere *et al.* [75], reported the concentration of sterol contents to be 5–9 mg/g and the concentration of resin acids to be 4–6 mg/g in pine (*Pinus sylvestris*) extracts prepared with ethanol and acetone by maceration at the boiling point. The obtained values of sterol content in pine bark (*Pinus sylvestris*) extracts closely match the reported values, while the obtained resin acid content is at the lower end of the referenced range. The lignan content in pine bark extracts is below the 4–6 mg/g commonly reported for Finnish pine bark, which may reflect differences in climatic zones [123]. Overall, these comparisons demonstrate that while the obtained concentrations of different compounds in pine extracts obtained by Soxhlet extraction are consistent with reported values in the literature, the values vary, likely due to the choice of solvent, the part of pine bark studied, and source-related factors such as season and storage conditions; as these changes can influence levels of terpenes, resin acids, and phenolics, while post-harvest conditions can accelerate the degradation of hydrophilic compounds and alter lipophilic fractions, especially in chipped material [124].

It should be noted that, the concentrations of fatty and resin acids obtained are significant because these odoriferous, bioactive compounds are widely known to be herbivore deterrents due to their bitter taste and digestion-inhibiting properties [2]. In contrast, steryl esters and sterols are less volatile but play a role in extract stability and film-forming capacity, which may increase persistence after application and reduce the requirement for re-treatment in the field [75,123]. Literature supports that pine bark extracts, more specifically, resin acids, phenolics, and terpenoids, are effective in mitigating browsing pressure [30]. These findings suggest that pine bark extracts synergise with quick-acting deterrent fractions (fatty and resin acids) and stabilising constituents (sterols and steryl

esters), making them an interesting candidate as bio-based actives for eco-friendly browsing repellent products.

4.2.5 Comparative analysis of birch and pine extras by Soxhlet extraction with EtOH

The comparative analysis of birch and Scots pine bark extracts shows clear differences in their extractability, chemical composition, and potential applications in browsing repellent formulations. Birch bark with natural low water content required minimal drying to provide efficient extraction, so maintaining thermolabile components such as phenolic acids and flavonoids. In contrast, pine bark contained a lot of water to begin with (~66%), and extensive drying was necessary to prevent solvent dilution and ensure effective recovery of resin acids and terpenoids.

Chemical analysis by spectroscopy also provided species-specific chemical fingerprints. UV–Vis spectra of birch extracts exhibited strong absorbance at 200–230 nm corresponding to triterpenoids such as betulin that are perfectly suitable for thermal extraction methods including Soxhlet extraction. FTIR spectra was also consistent with such interpretations, with birch extract showing bands characteristic of pentacyclic triterpene (e.g., exomethylene band at 887 cm^{-1}) and free carboxylic acids, but pine extracts showing more intense ester carbonyl bands at $\sim 1730\text{ cm}^{-1}$, rich aliphatic C–H stretches, and lignin markers, reflecting a composition dominated by resin acids, fatty acids, and esterified lipids.

Such differences are directly relevant for browsing repellent formulation. Birch bark extract, due to high concentrations of bitter and unpalatable triterpenoids, would work primarily as a taste-based repellent. Its hydrophobic nature and chemical stability also suggest persistence on plant surfaces, contributing to long-term protection. Pine bark extract has high resin acid and volatile terpene concentrations that give strong odour-based deterrent properties.

4.3 Characterisation of sheep wool grease

Sheep grease was characterised alongside HPA lanolin, commercial Trico repellent, and sheep fat to compare their chemical composition and functional groups. The analysis focused on identifying lipid classes and bioactive compounds relevant to repellent activity.

4.3.1 Lipid class analysis by high-performance thin-layer chromatography (HPTLC) of sheep wool extracts and reference samples

Lipid class distribution of the different extracts of wools from Finnsheep, Texel sheep and reference samples was analysed using high-performance thin-layer chromatography (HPTLC). This method enabled the quantification of major lipid fractions, including free cholesterol, fatty alcohols, free fatty acids, triacylglycerols, diesters, wax esters, and cholesteryl esters. The relative proportions of these lipid classes (expressed as weight percentage) are presented in Figure 26 for HPA lanolin, sheep fat, the commercial repellent Trico, and wool extracts obtained from Finnsheep and Texel sheep.

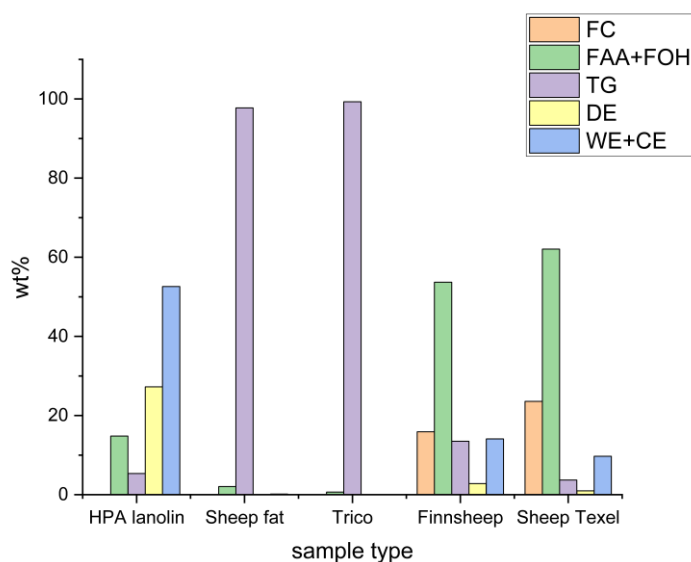


Figure 23. Lipid class composition (wt %) of highly purified anhydrous (HPA) lanolin, sheep fat, Trico repellent, and extracts of wools from Finnsheep and Texel sheep, as determined by high-performance thin-layer chromatography (HPTLC). FC = free cholesterol; FFA = free fatty acids; FOH = fatty alcohols; TG = triacylglycerols; DE = diesters; WE = wax esters; CE = cholesteryl esters.

According to the results obtained by HPTLC analysis, HPA lanolin demonstrated a profile dominated by wax ester and cholesteryl ester (WE+CE, ~50 wt%), along with diesters (DE, ~25 wt%), free cholesterol (FC, ~15 wt%), and minor amounts of free fatty acids and fatty alcohols (FFA+FOH, ~10 wt%). The obtained results are consistent with Sengupta *et al.*'s review [32], which reported that purified lanolin typically consists of 35–45% wax esters, 11–14% sterols, and varying amounts of fatty alcohols and free acids, giving lanolin hydrophobic and protective properties.

Texel and Finnsheep wool grease extracts obtained by Soxhlet extraction in EtOH contained a broader distribution of lipids compared to purified lanolin. Texel sheep extract

consisted mainly of FFA+FOH (~60 wt%), then FC (~20 wt%) and WE+CE (~10 wt%), while Finnsheep wool grease extract contained ~55 wt% FFA+FOH, ~15 wt% WE+CE, and ~10–15 wt% FC. These FFA and sterol concentrations are higher than the purified standards, which concur with crude wool grease of Soxhlet extraction, wherein insufficient purification leads to the preservation more refined combinations of free alcohols and lipids, as El sayed *et al.* [70], described in their review. Similarly, López-Mesas *et al.* [92], reported that raw wool wax contained 20–40% FFA, sterols, and fatty alcohols, in contrast to the purified profile of lanolin. Sheep fat, for comparison, was dominated by triacylglycerols (TG > 95 wt%), a characteristic of lipids in adipose tissues [125].

On the other hand, the commercial repellent Trico contained near-pure TG (~100 wt%) with negligible contributions from sterols, fatty acids, or wax esters. This is quite different from sheep wool extracts that contained bioactive sterols, fatty alcohols, and wax esters. Functionally, this distinction is critical: whereas Trico's deterrence is primarily olfactory, based on smell signals from ruptured triglycerides [7], wool grease extracts may combine odour-based deterrence with film-forming hydrophobicity and lasting residence on leaf surfaces due to their higher steryl ester and wax ester composition [32].

The presence of sterols and steryl esters in wool grease extracts not only contributes to hydrophobicity, but also enhances stability and film-forming capacity, which may prolong repellent efficacy after application and reduce re-application frequencies. Lanolin and wool grease have also been used for their adhesive, protective, and water-repellent properties in cosmetic and industrial applications for centuries [70,92], indicating the same promise in forestry repellents to deter browsing herbivores. In addition, wool grease (lanolin) is a natural animal-derived material and is widely utilised and accepted in EU industries, including pharmaceuticals and cosmetics, when impurities such as suint residues and pesticides are removed [126]. Therefore, sheep wool extracts are a regulation-compliant, biodegradable, and functionally improved raw material for repellent development.

5. Conclusions

This study demonstrates that cruciferous vegetables, bark residues, and wool grease from sheep can be valorised as locally available raw materials in the development of natural browsing repellents. Sulphur-rich cruciferous biomass provides immediate odour deterrence. Birch and pine bark contribute bitter and resinous defence compounds. Wool grease enhances adhesion and persistence properties. The results presented in this work highlight the potential of these biomasses for the formulation of an effective, long-lasting, and sustainable browsing repellent against moose and deer.

The key findings of this study are summarised as follows:

- The most promising cruciferous biomass was broccoli. It had a strong sulphurous odour and the highest UV absorbance at 196 nm and 227 nm. These findings are potentially consistent with high sulforaphane and glucosinolate contents, as demonstrated by FTIR analysis. The highest glucosinolate peaks in the region of 1630-1690 cm^{-1} were observed for the optimised extraction procedure, in which broccoli was pretreated by freezing and extracted using UAE in water at 50 °C for 20 min. Based on the results, pretreatment by freezing likely enhanced the release of sulphur-containing metabolites such as sulforaphane and indole-3-carbinol. Although red cabbage showed less intense sulphur signals compared to broccoli and cabbage extracts, it contained the highest concentrations of TPC. This is in agreement with the known high anthocyanin and phenolic content of red cabbage (scopes 1 and 2).
- Birch bark possessed low moisture content compared to pine bark (6 % and 66 %, respectively). Birch bark extract obtained by Soxhlet extraction with EtOH was rich in triterpenoids such as betulin and betulinic acid. This was identified by FTIR peaks at 887 cm^{-1} assigned to exomethylene band and UV absorbance between 200–230 nm assigned to betulin. These hydrophobic, bitter chemicals act as taste-based deterrents with phytogenic persistence on plant surfaces. Moreover, birch bark has a distinct white coloration that functions as a visual deterrent to browsing mammals such as deer and moose. On the other hand, Scots pine bark extract contained volatiles responsible for odour-based deterrence. These are characterised by resin

acids, fatty acids, and esterified lipids, as indicated by ester carbonyl peak at around $\sim 1730\text{ cm}^{-1}$ in FTIR spectra. GC-MS results showed that lipophilics and lignin residuals were the dominant extractive fractions ($\approx 64\text{ mg/g}$ and 34 mg/g , respectively) (scopes 1 and 2).

- HPTLC analysis of extracts of sheep wool by Soxhlet extraction in EtOH indicated that Finnsheep and Texel sheep wool grease contained around $\sim 55\text{--}60\text{ wt\%}$ free fatty alcohols and acids, $\sim 10\text{--}20\text{ wt\%}$ free cholesterol, and $10\text{--}15\text{ wt\%}$ wax and cholesteryl esters. In contrast, purified lanolin contained mostly esters, diesters, and cholesterol ($\sim 50\text{ wt\%}$, 25 wt\% , and 15 wt\% , respectively), and commercial repellent Trico, which is based on sheep fat, consisted of only triglycerides (100 wt\%). Thus, Trico relies on odour signals from triglyceride degradation, whereas wool grease extracts have odour deterrence as well as film-forming properties due to wax and sterol esters, providing possible persistence for a longer time after deposition (scopes 1 and 2).
- The researched biomasses, cruciferous vegetables, pine and birch bark, sheep wool from existing breads, are widely accessible in Finland as by-products of forestry and agriculture. They have been widely used and accepted in EU industries (e.g., pharmaceuticals, cosmetics), which indicates compliance with regulation and environmental safety (scope 4).

Nevertheless, this study has limitations. Extraction and characterisation were restricted to laboratory conditions and small-scale trials, without long-term stability testing or field validation under variable Nordic climates. The environmental impact assessment was qualitative rather than quantitative, and scalability remains to be tested. Reflecting on the process, expanding the dataset to include seasonal variation, longer extraction optimisation trials, and larger field experiments would have strengthened the conclusions.

5.1. Future perspectives

Based on the results of this study, several research directions are outlined to continue the current work on cruciferous biomass, bark extracts, and sheep wool grease as natural browsing repellent components:

- Quantitative profiling of bioactive compounds such as sulforaphane and glucosinolates of cruciferous biomass, and triterpenoids of birch carried out using

the UHPLC and LC-MS techniques to determine their precise concentrations and identify the threshold concentrations required for effective repellence formulations.

- Optimisation of extraction methods to define the optimal yields, the effect of temperature range of 50 °C on glucosinolates, as well as the effect of deep-freezing (at -80°C) treatment prior to extraction, cell disruption, glucosinolate hydrolysis, and sulforaphane release from cruciferous biomass. The extraction optimisation should also include testing other extraction methods (e.g., supercritical CO₂ and microwave-assisted extraction).
- Synergy tests and formulation development blend cruciferous, bark, and wool grease extracts into formulated repellent systems to study their complementary properties (sulphur volatiles for odour, triterpenoids and resin acids for taste/odour, and wax esters for persistence). Further incorporation of extracts into carriers such as emulsions, biodegradable polymers, or wax coatings might enhance further adhesion and stability of browsing repellent.
- Field trials under Nordic climate conditions are essential to test the efficacy of repellents against moose and deer browsing, and to measure the long-lasting effect under seasonal variations.
- Comparisons with other synthetic (e.g., thiram-based repellents) and natural repellents (e.g., Invisideer, chilli pepper extracts, Trico, and garlic extracts) is also a key factor in evaluating the formulated repellent's effectiveness.

Supplementary materials

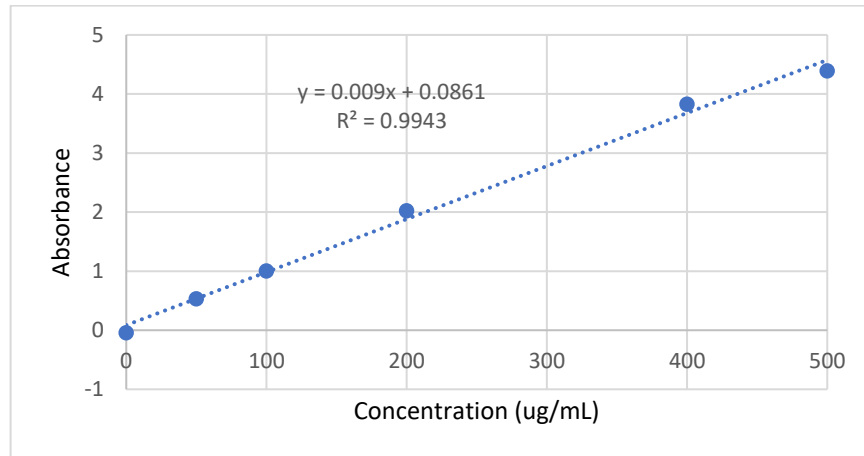


Figure S 1. Calibration curve of gallic acid for total phenol content determination.

Table S 1. Aligned Rank Transform ANOVA summary for Model 2 assessing the effects of biomass, extraction method, and temperature on total phenolic content

Term	Df	Sum_Sq	F_value	p_value	Significance	Partial_Eta2	Magnitude
Biomass	2	3456.000	99.69231	2.365352e-12	***	0.8926	Large
Method	1	2916.000	74.87589	7.654653e-09	***	0.7573	Large
Temperature	1	2916.000	74.37194	8.144752e-09	***	0.7560	Large
Biomass:Method	2	3502.722	116.75741	4.294457e-13	***	0.9068	Large
Biomass:Temperature	2	3547.056	136.42521	7.799787e-14	***	0.9192	Large
Method:Temperature	1	2916.000	76.34618	6.398535e-09	***	0.7608	Large
Biomass:Method:Temperature	2	3472.056	106.28741	1.188263e-12	***	0.8986	Large

Table S 2. Aligned Rank Transform ANOVA summary for Model 3 evaluating the effects of biomass and temperature on total phenolic content with ethanol + ultrasound-assisted extractions.

Term	Df	Sum_Sq	F_value	p_value	Significance	Partial_Eta2	Magnitude
Biomass	2	432.0000	52.18792	1.202017e-06	***	0.8969	Large
Temperature	1	364.5000	38.59412	4.501577e-05	***	0.7628	Large
Biomass:Temperature	2	394.3333	26.78491	3.757196e-05	***	0.8170	Large

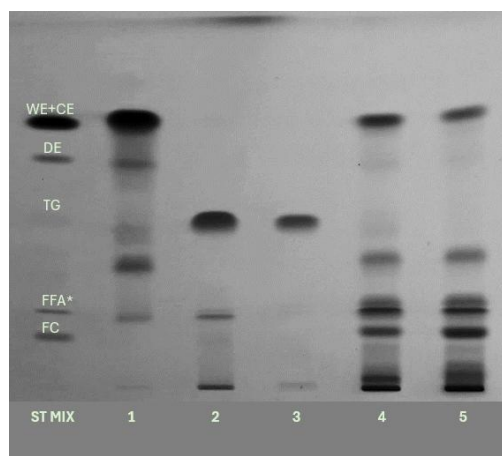


Figure S 2. High-performance thin-layer chromatography analysis of lipid classes in different samples: (1) HPA lanolin, (2) sheep fat, (3) Trico, (4) Finnish sheep wool extract (Saimas), and (5) Finnish sheep wool extract (Texel). Where; free cholesterol (FC), fatty alcohols (FOH), free fatty acids (FFA), triacylglycerols (TG), diesters (DE), wax esters (WE), and cholesteryl esters (CE).

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