Application of horse chestnut saponin as an alternative antimicrobial agent in soaking process*

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Abstract

Soaking process is carried out with synthetic surfactants including bactericides, salts and enzymes or chemicals formulated as a mixture of surfactant, bactericide and enzyme preparations in the form of aqueous emulsions. In this research, the feasibility of potential application of aescin, a plant-derived saponin from horse chestnut seed, as an alternative natural antimicrobial biosurfactant in soaking process was investigated in terms of microbial load, total protein content and chemical oxygen demand of soaking residual floats. Soaking baths were treated using commercial product of aescin saponin with 98% purity, and a synthetic bactericide. The antimicrobial activity of aescin saponin was tested by applying different concentrations (0.125, 0.25, 0.5 and 1g) based on sapogenin content of aescin commercial product. Application of horse chestnut saponin provided comparable results to synthetic bactericide with prolonged soaking time. Increased total protein content of soaking liquor accompanied by decreased microbial growth was a good sign of antimicrobial soaking characteristic of aescin saponin. The results revealed that aescin showed a concentration-dependent response and has the potential to be a natural alternative to synthetic surfactants and antimicrobial compounds for leather industry.

Keywords: horse chestnut saponin, aescin, soaking, antimicrobial.

Introduction

In recent years, studies regarding the investigation of alternative and environmentally friendly products used in leather processing steps for the replacement of polluting chemicals have gained significant interest with the effect of stricter environmental legislations and increasing concern over environmental pollution. Leather industry utilizes wide variety of chemicals in the production steps, and known as a branch of industry with the prompt application of new, environmentally friendly products and innovative techniques. In this context, biosurfactants have an important potential use in terms of being a resource for environmentally friendly products that can be applied in multiple leather production processes like soaking and degreasing [1-4] where synthetic surfactants are used in conventional processes. Besides, investigation of the potential applications of plant-derived biosurfactants in leather processing steps as an alternative to chemical surfactants is of considerable importance due to heavy pollution load of the leather industry.

Biosurfactants are surface-active biomolecules that have a great potential in numerous application when compared to chemical surfactants, due to having several advantages such as low toxicity, high biodegradability, ecological acceptability, biocompatibility, and durability to extreme process conditions [1, 5-8]. These distinctive properties of biosurfactants can provide advantages to leather industry, which utilize great quantities of chemical surfactants and closely monitored in terms of its polluting capacity.

Saponins are a structurally diverse group of plant derived biosurfactants, which are naturally occurring surface-active glycosides and widely distributed in the plant kingdom such as soybeans, chickpeas,

alfalfa, navy, mung, and kidney beans, horse chestnut, licorice, Mojave yucca and Soap bark tree [1, 4, 9, 10]. These natural surface-active compounds are known as a capable of reducing surface and interfacial tension between different fluid phases based upon their water and fat-soluble components [4, 7, 11]. Saponins have pharmacological and medicinal properties [12] as well as their foaming and emulsifying properties [13], and they also exhibit antimicrobial, antioxidant, insecticidal, and molluscicidal activities [14, 15]. They are generally used in food, agricultural, cosmetics and pharmaceutical industry [9]. However there have been very few studies conducted on feasibility of using plant-derived biosurfactants in leather manufacturing processes. Kılıç reported possible application of the quillaja saponins as degreasing agents [2], Adıgüzel Zengin (2013) utilized quillaja as antimicrobial soaking agent [1] and Zengin et al., investigated possible application of aescin saponin, a plant derived biosurfactant from horse chestnut seed, as an alternative degreasing agent [4].

To the best of our knowledge up to date there has been no scientific research reported regarding application of aescin saponin as an antimicrobial soaking agent for leather industry. In order to fill the gap it was aimed to investigate whether aescin has an inhibitory effect on microbial load of soaking liquor.

Materials and Methods

Aescin is commercially available in white powdered form with 98% of sapogenin content Carbosynth (Berkshire, UK). It is entirely soluble in distilled water and used without further purification. Possible antimicrobial effects of the aescin solutions were tested against commercial bactericides using raw, wet-dry salted metis type sheepskins. A blank control assay was also performed without addition of aescin or bactericides. Sheepskins were cut into 10x10 cm square pieces and pre-soaked under static conditions for 2 hours in 1:20 water, based on skin weight. Aescin solutions containing different concentrations of sapogenin (0.125, 0.25, 0.5 and 1g) were prepared using 30ml distilled water. Commercial bactericide based on benzisothiazolinon, was employed according to recommended dosage level ($0.1gL^{-1}$) of the manufacturer [1].

Aescin solutions, and commercial bactericide were added to the soaking bath (1:20) for each assay and the soaking process was performed up to 24 h. The assays were done in duplicates and the results were given as mean values.

The bacterial load of the soaking baths was determined with the samples taken at 8th and 24th hours of the process by the use of the tube dilution plate method. Serial dilutions of samples were prepared and plated on Plate Count Agar (Merck, Darmstadt, Germany). The agar plates were incubated for 24h in an incubator at 37^{o}C and colony-forming units (cfu) were counted according to the method described in the literature [1, 16]. The total protein content of the soaking baths was analyzed according to Bradford, 1976 [17]. The COD of the spent liquor from the soaking operation was measured with standard kits (Merck) with Merck Move 100 Water and Wastewater Spectrophotometer.

Results and Discussion

The inhibitory efficiency of the horse chestnut saponin, aescin, with different sapogenin contents on microbial population of soaking liquor was expressed as the colony forming units determined within 8 and 24 hours of the soaking process. Figure 1 gives the results of microbial growth for each soaking assay. The results of colony forming units show that the inhibition effect of aescin saponin for 8 hour soaking time remains nearly unchanged with the increasing saponin concentrations (Fig. 1). The microbial load of the soaking processes treated with increasing aescin concentrations for 8 h were reduced up to 10% compared to blank trials, which can be considered as a good sign of better protection of aescin in comparison to other plant derived saponins reported in the literature [1].

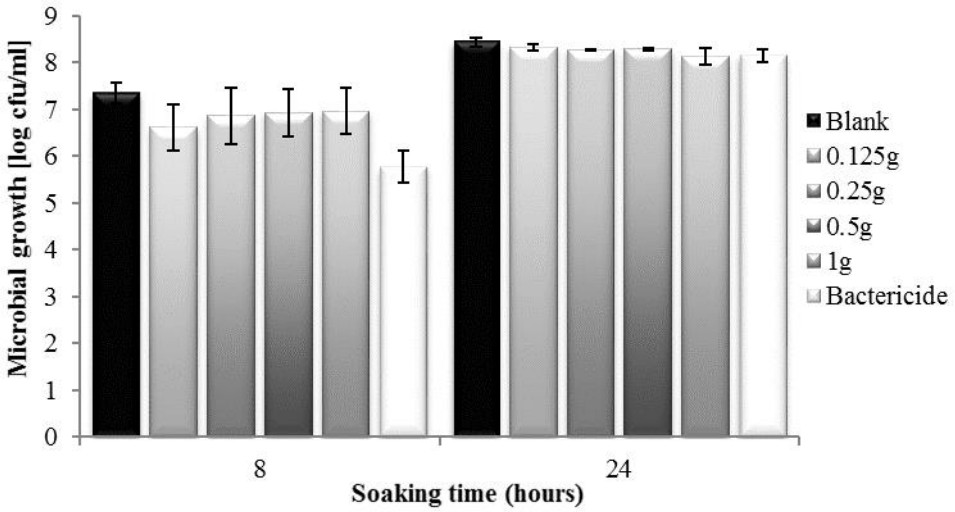

Figure 1 Effect of increasing aescin concentration on microbial growth of soaking liquor determined as log cfu/ml (Data are presented as mean ± standard error of the mean)

A slight decrease was observed for microbial load of soaking baths that could be attributed to a concentration dependent response for aescin treatments after 24 hours of incubation. Commercial bactericide provided higher efficiencies up to 20% with 8 hours of soaking process. However with the prolonged soaking time of 24 hours, inhibitory effect of bactericide was significantly reduced to 3.4%, where highest aescin concentration showed 3.7% reduction in microbial growth. Better antimicrobial protection was achieved in comparison to quillaja saponins [1].

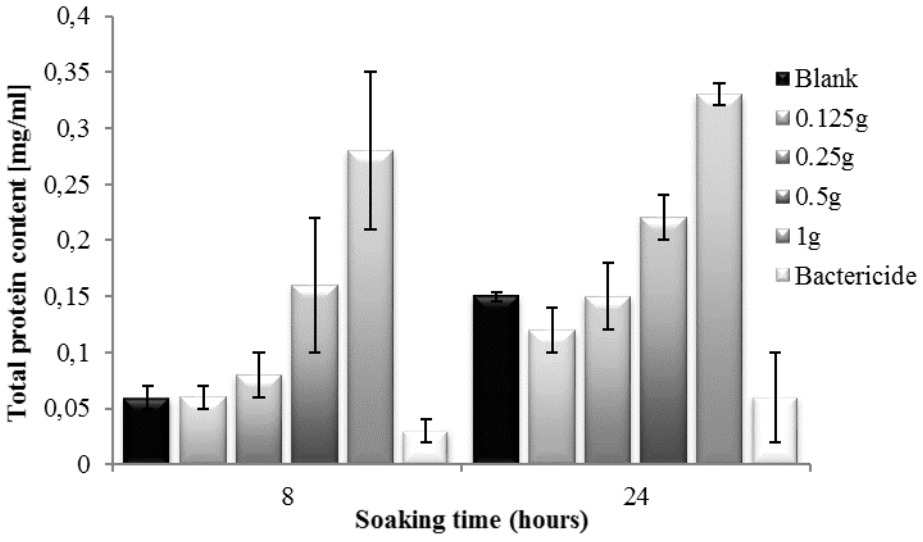

Figure 2 Total protein content of soaking liquors treated with aescin and other commercial agents by Bradford method (mg/ml)


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Total protein content (mg/ml) of soaking liquors treated with aescin and commercial bactericide are shown in Figure 2. The protein content of soaking liquors was increased with increasing aescin concentration levels and findings are in line with the early work performed by Adiguzel Zengin (2013). The microbial population of these soaking liquors was inhibited by the use of increased aescin concentrations. These findings especially are a well indicator of the potential soaking efficiency of aescin saponin accompanied by its antimicrobial activity.

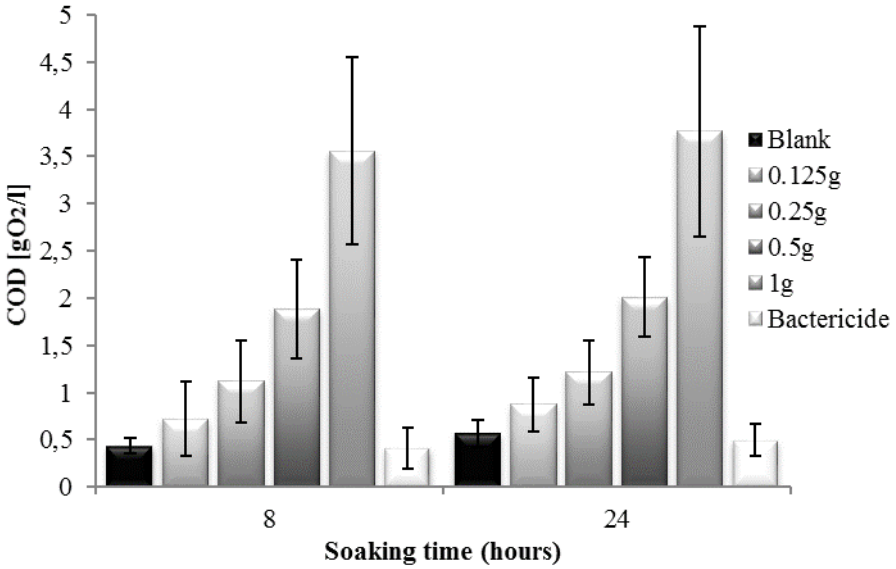

Figure 3. Comparison of COD values of soaking liquor at 8th and 24th hours of the process (gO_2/l)

The COD results of the soaking liquors treated with aescin saponin were presented in Fig. 3. Aescin treated soaking liquors have significantly higher COD values than the blank trials and synthetic antimicrobial agent applications. A concentration dependent response was achieved, where application of 1g aescin gave the highest COD value, 3.7 gO_2/l. These findings supports the previously published results [18].

Conclusion

This paper analyzes the feasibility of using aescin, a plant-derived saponin from horse chestnut seed, as an alternative natural antimicrobial biosurfactant in soaking process. The effect of aescin was investigated in terms of microbial load, total protein content and chemical oxygen demand of soaking residual floats and following conclusions have been drawn. Application of highest concentration of aescin sapogenin content (1g) with 24h of soaking time showed positive effect on decreasing the microbial load of the soaking liquors and provided a comparable antimicrobial effect to that of commercial bactericide. Increased total protein content of soaking liquor accompanied by decreased microbial growth was a good sign of antimicrobial soaking characteristics of aescin saponin. Considering the unique properties of plant derived biosurfactants, and findings from this study it can be concluded that aescin has the potential to be an alternative to synthetic surfactants and antimicrobial agents for leather industry.

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