British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.1372

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

Proceedings of the 14th APS International PharmSci 2023

An Assessment of Pectin Thiolation parameters: Degree of Esterification, Conjugate and Cross-linking Reagent Concentrations

Jack Phillip Brown[a], Dr Adeola Adebisi[b], Professor Barbara Conway[a]*

[a]University of Huddersfield , Queens Gate, Huddersfield HD1 3DH, United Kingdom

ARTICLE INFO

Received: 09/06/2023
Revised: 19/06/2023
Published: 31/12/2023

*Corresponding author.
Tel.: +99 1234 567 890
E-mail: a.b@xyz.ac.uk

KEYWORDS: Pectin; L-Cysteine; Thiomer; Degree of Esterification

SUMMARY

This study focuses on the optimisation of pectin thiolation using L-cysteine and assesses the impact of the degree of esterification (DE), polymer to L-cysteine ratio, and concentrations of conjugating reagents on the total and free thiol content of highly esterified (HE) and lowly esterified (LE) pectin thiomers. The thiolation process was analysed under variations of four factors - the type of pectin used (HE and LE), the polymer to cysteine ratio, and concentrations of the conjugating reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) and N-hydroxysuccinamide (NHS) (0 mM, 25 mM, 50 mM & 100 mM). Thiol content was determined using Ellman's reagent, with spectrophotometric methods. Statistical analysis via multivariant analysis of variation (MANOVA) indicates a significant relationship with respect to total thiol content considering DE, polymer to cysteine ratio, and EDAC concentration ($P<0.05$). Concordantly, MANOVA indicated a significant relationship with respect to free thiol content considering DE, polymer to cysteine ratio, and both EDAC and NHS concentrations ($P<0.05$). Increasing EDAC concentration corresponded with increased total and free thiol content in both types of pectin. In contrast, elevating NHS concentration led to reduced total thiol content across most ratios. Thus, the study delineates critical parameters in the thiolation of pectin using L-cysteine.

INTRODUCTION

The thiolation of pectin has been covered by several authors with the aim of enhancing the tensile properties of pectin (Hintzen et al. 2013; Knoll et al. 2021). The aim of this study was to optimise the thiolation of LE and HE pectin using L-cysteine as the thiolating agent and to determine the effect of DE, polymer: L-cysteine ratio, and conjugating reagent concentration on the total and free thiol content of these polymers.

MATERIALS AND METHODS

PECTIN THIOLATION: Briefly, pectin was hydrated in deionised water overnight at room temperature. To activate the carboxyl groups on the pectin backbone a specified amount of EDAC and NHS, were dissolved into the hydrated pectin solution in the absence of light at room temperature for 1 hour. A predetermined amount of L-cysteine was dissolved into this polymer solution and this mixture was agitated in the absence of light for 3 hours. The pH of the polymer solution was maintained at 4.5. The pectin-cysteine solution was purified by dialysis. The dialysed solutions were frozen at -20 °C and freeze-dried (Christ Alpha 2-4 LD plus, manufacturer Germany) at -40 °C and 0.120 mbar.

DETERMINATION OF FREE THIOL CONTENT: Free thiol content was measured via a colourmetric assay. Thiolated pectin was hydrated with sodium phosphate buffer. To the sample, 5-5'-dithiobis-(2-nitrobenzoic acid) was infused and left for 45 minutes. The absorbance of this sample was determined at 412 nm using a UV spectrophotometer (Jenway 7305, UK).

DETERMINATION OF TOTAL THIOL CONTENT: Total thiol content was also determined using Ellman's reagent. Thiolated pectin was hydrated in sodium phosphate buffer and sodium borohydride This mixture was incubated at 20 °C for 1 hour. HCl

https://doi.org/10.5920/bjpharm.1372

(5 M) was aliquoted into the mixture and left for 10 minutes. Sodium phosphate buffer and Ellman's reagent were added to the mixture and incubated at 20 °C for 2 hours. After 2 hours the UV absorbance of the mixture was measured at 412 nm (Perrone et al. 2017).

RESULTS AND DISCUSSION

THIOL CONTENT, EDAC, NHS AND PECTIN-CYSTINE RATIOS: FT and TT contents of samples are summarised in Fig. 1 and Fig. 2. Data from a multivariate analysis of variance (MANOVA) suggests DE, pectin-cysteine ratio and EDAC concentration significantly influence the total thiol content ($P<0.05$). With respect to free thiol content, DE, pectin-cysteine ratio, EDAC and NHS concentrations are significant influences ($P<0.05$).

THIOL CONTENT: DEGREE OF ESTERIFICATION: DE significantly influenced the FT and TF content of thiolated pectins (95 % confidence interval (CI) [27.51-57.5], [-193.2 - -62.4] $P<0.05$ respectively). Overall, the TT content of HE pectin samples was higher than that of LE pectin samples ($p<0.05$).

THIOL CONTENT: EDAC CONCENTRATION: In aggregate, with respect to pectin: cysteine ratio and DE, TT and FT content of pectin samples increased concordantly with EDAC concentration ($P<0.05$) (Fig. 1.).

THIOL CONTENT: NHS CONCENTRATION: TT content of both HE and LE samples decreased across all samples when NHS concentration was increased, with the exception of 1:0.5 (HE) 0 mM vs 1:0.5 (HE) 100 mM which showed a 121.3% increase in TT content.

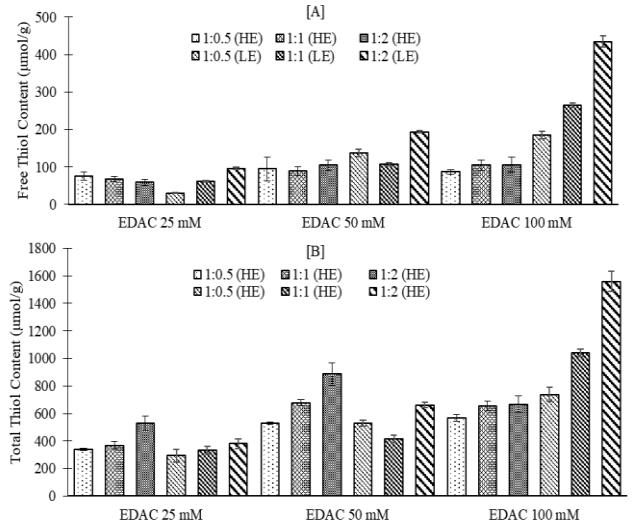

Fig. 1. *[A] Free thiol content (µmoles g-1) of HE and LE thiolated pectin samples exposed to varying concentrations of EDAC and L-cystine in presence of 50 mM NHS. [B] Total thiol content (µmoles g-1) of HE and LE thiolated pectin samples exposed to varying concentrations of EDAC and L-cysteine in the presence of 50 mM NHS.*

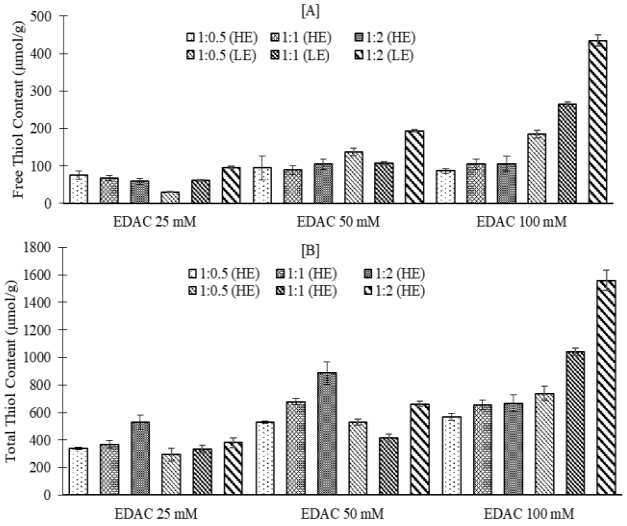

Fig. 2. *Total (TT) and free (FT) thiol content (µmoles g-1) of HE and LE thiolated pectin samples exposed to varying concentrations of NHS and L-cystine in presence of 50 mM EDAC.*

CONCLUSIONS

Thiolated samples were modified to levels concordant to literature and characterised in terms of free thiol and mean oxidised thiol content. L-cysteine, EDAC and NHS concentration, in conjunction with DE were found to be significant influences with respect to free and total thiol content.

ACKNOWLEDGEMENTS

I would like to thank Dr Adeola Adebisi for sharing her extensive expertise and guidance, Professor Barbara Conway for her sustained endeavour to further develop and advance the research capabilities of our department.

REFERENCES

Hintzen et al., 2012., Drug Dev Ind Pharm, 38: 1479-85.

Hintzen et al., 2013. Eur J Pharm Biopharm, 85(3B):1266-73

Knoll et al., 2021. Acta Biomaterialia, 135: 139-49.

Perrone et al., 2017. Eur J Pharm Biopharm, 115: 168-76.

Suchaoin et al., 2016. Int J Pharm, 503: 141-9.