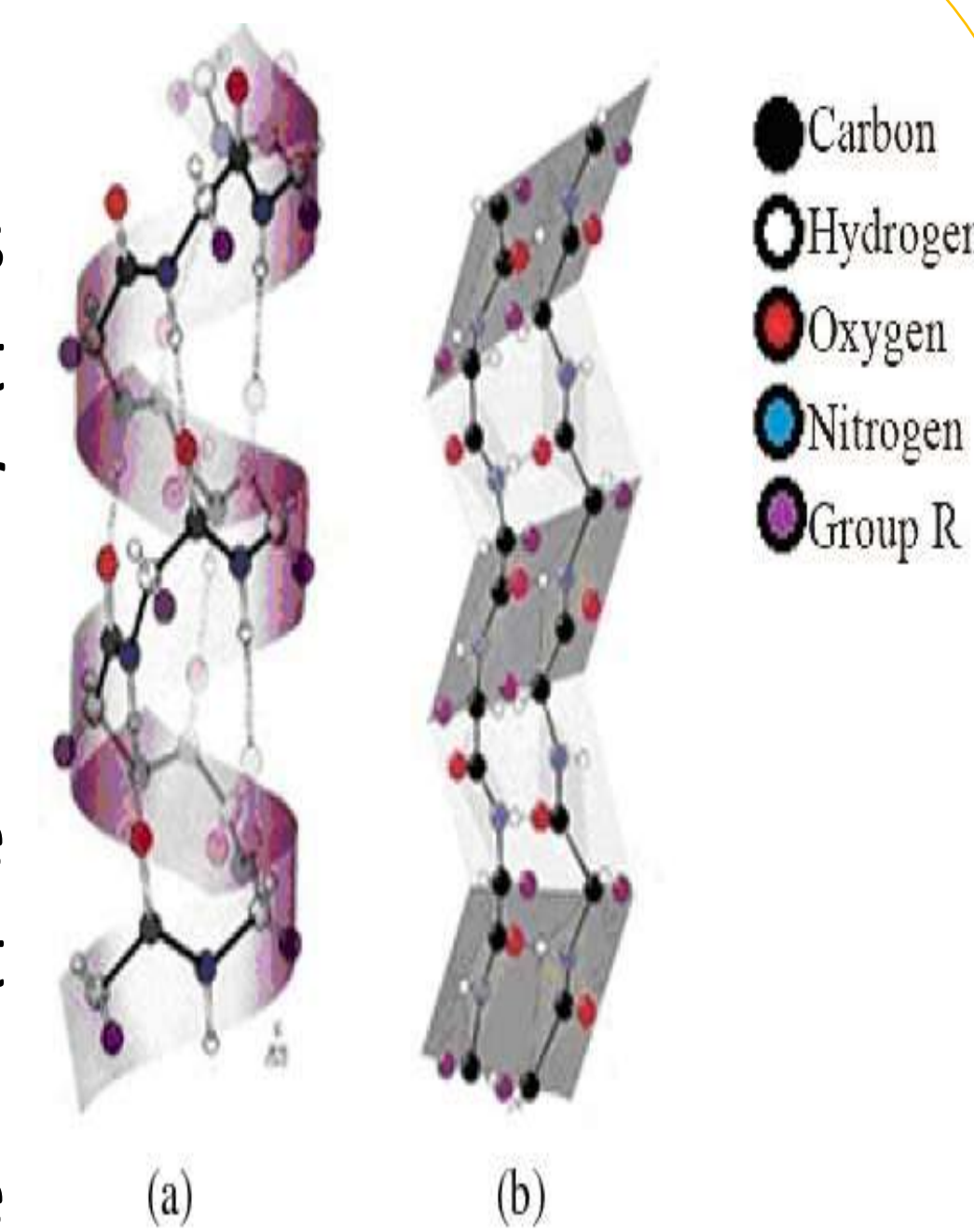


## Introduction

- Chicken feather waste generated from poultry industry is increasing rapidly. Every year the amount of poultry meat produces is around 100.5 million tons as a result, the feather waste generated around the world > 4.7 million tons.
- The poultry feathers are a low-cost by-product and utilization of this agricultural waste is intended to reduce the impact on environment. Poultry feathers – excellent source of keratin (>90%).
- Keratin is a biopolymer with extensive potential to be shaped into wound healing biomaterials. Extracting keratin from poultry feathers is aimed to reduce the accumulation of landfills generating an opportunity to make additional profits in the poultry industry. Further, use of this waste is a promising environmentally sustainable technique considering going towards green.
- Keratin is biocompatible and less-cytotoxic due to the presence of cell recognition sites (ex: leucine-aspartic acid-valine and arginine-glycine-aspartic acid that promotes cell attachment and proliferation).



**Keratin structure**  
(a)  $\alpha$  helix, (b)  $\beta$  helix (Image source: Belarmino., et al 2012)

(Adams, and Watt, 1989; Belarmino et al., 2012; Esparza et al., 2018; Paul, 2015; Gupta et al., 2011; Smithers et al., 2016)

## Hypothesis

- Fabricating of scaffolds using biomimetically modified keratin with Tyrosinase and polymer blending with chitosan will improve the mechanical properties of wound dressings/ scaffolds.

## Objectives

- To improve mechanical properties of the keratin biomaterial by polymer collaboration with tyrosinase and chitosan rich mussel extracts.

## Materials and Methods

### Modification of Keratin

Keratin - dispersed in de-ionized water (5% w/v)

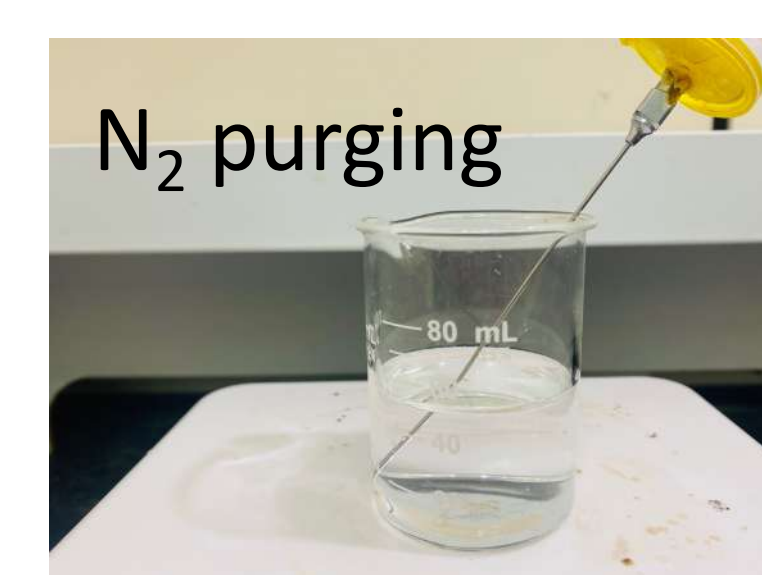
pH - adjusted to 7

Allow for react with continued stirring (30m/ 250 rpm)

Solutions were purged with N<sub>2</sub> gas (15 m; 300 rpm; 25 °C)



pH - adjusted to 7



## Materials and Methods

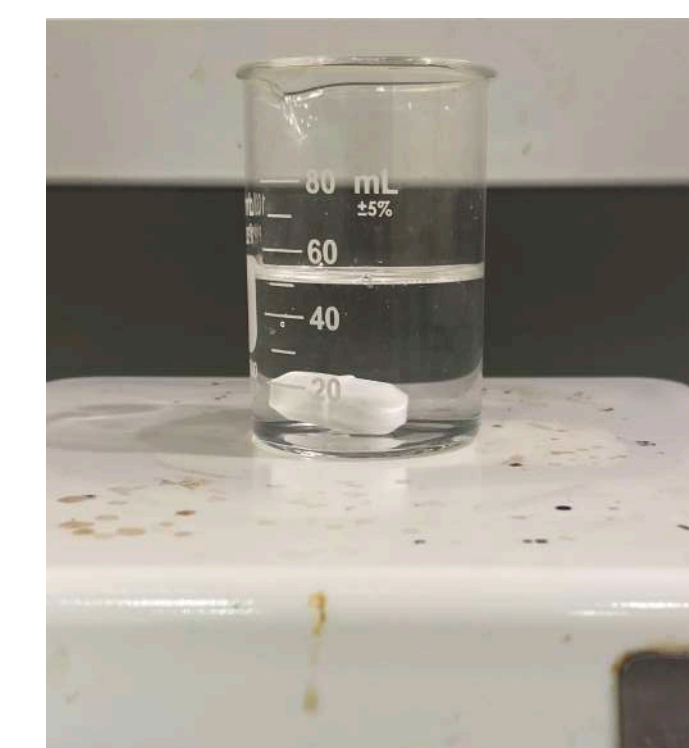
### Modification of Keratin

Tyrosinase enzyme - added to keratin dispersion at a ratio of 50 $\mu$ g/gm (tyrosinase: keratin)

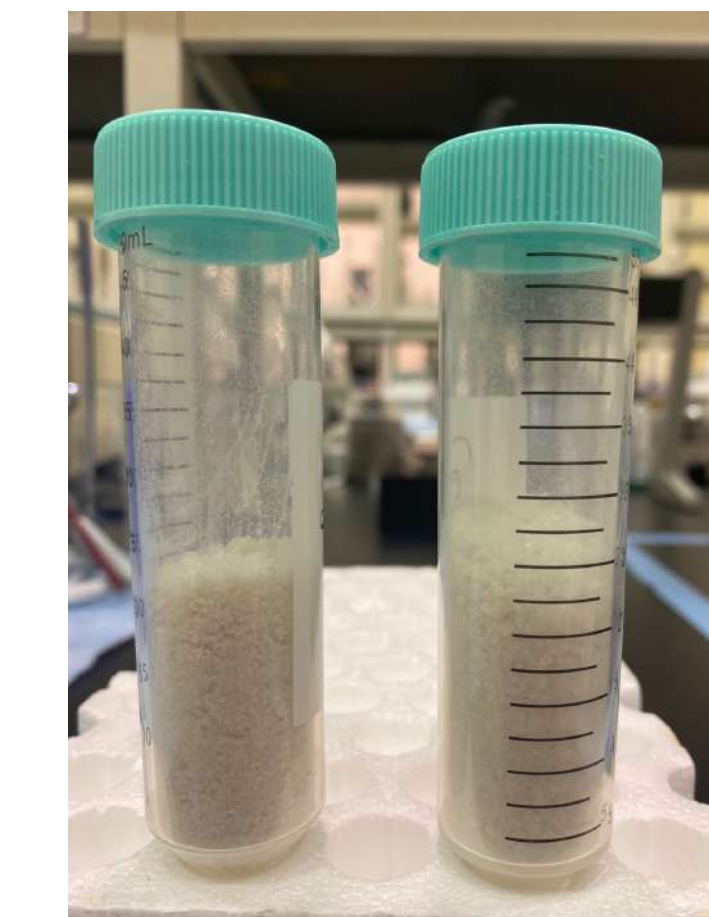
Stir for 2h under continuous N<sub>2</sub> purging (250 rpm; 25 °C)

After 4 h, reaction was terminated

Solution was frozen at -20 °C



Stir for 2h



Modified Keratin

### Fabrication of Scaffolds

50ml of 1% acetic acid solution was heated at 70°C

Chitosan was added in the above solution and stirred at 350 rpm/ 30 min

Keratin powders (modified/ unmodified) added at 0%, 10%, 20%, 30% 40%, and 50% w/w ratios of chitosan and stirred at 350 rpm/ 60 min

The final mixture was filled into 6ml syringe and transferred into polytetrafluoroethylene (PTFE) ring molds

Samples were lyophilized and characterized for surface hydrophobicity, morphology, mechanical strength, chemical properties, thermal stability, and impact of the modification on crystallinity

## Results

Table 01: Surface Hydrophobicity of keratin and modified keratin.

Keratin sample	So
Unmodified Keratin	518.96
Modified Keratin	743.65

Surface Hydrophobicity of unmodified keratin and modified keratin was measured using ANS probe method. The test results showed that the surface hydrophobicity was increased in modified keratin (MK) than that of unmodified keratin (UK). This could be due to the biomimetic modification that took place in the modified keratin samples.

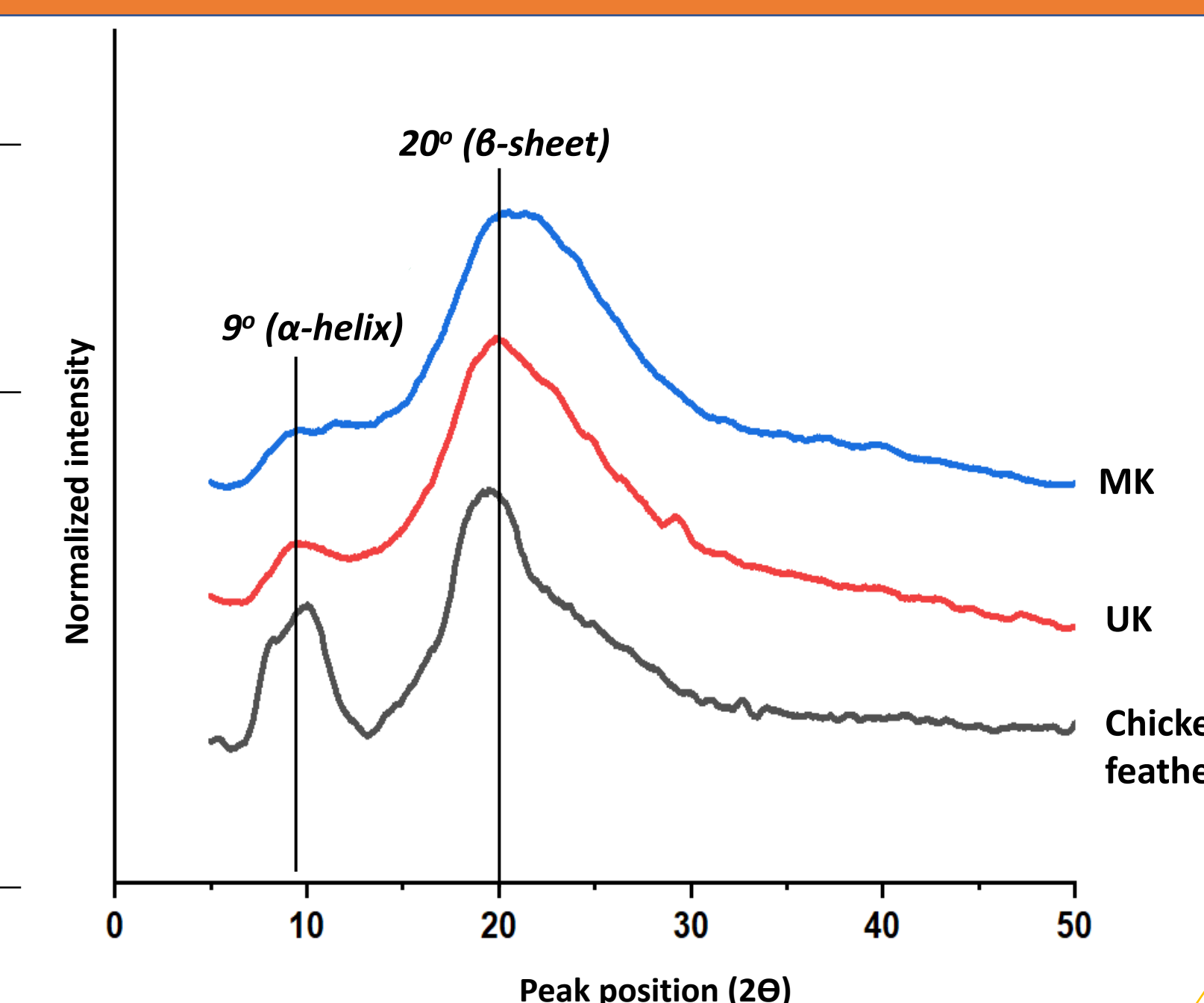


Figure 01: Higher amounts of  $\alpha$ -helix and  $\beta$ -sheets were detected for MK scaffolds from the significantly higher XRD peaks at 2 $\theta$  value of 9° ( $\alpha$ -helix) and 20° ( $\beta$ -sheet).

## Results

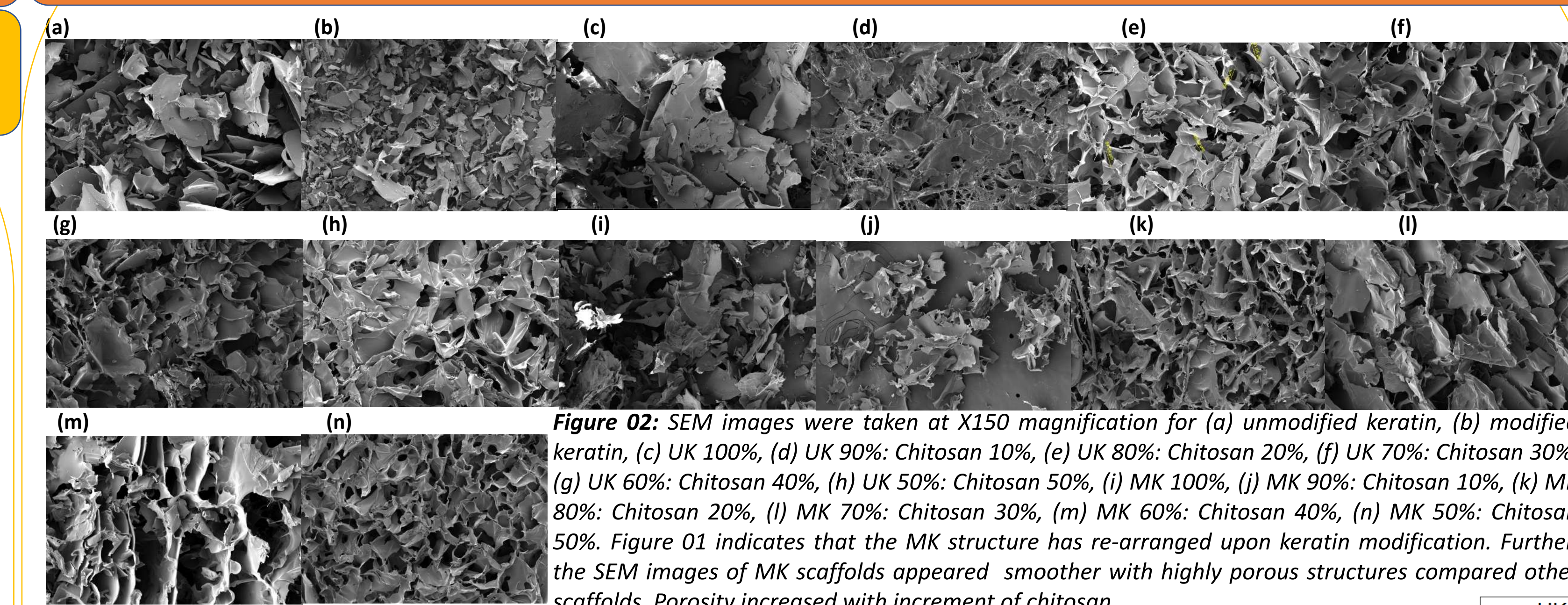


Figure 02: SEM images were taken at X150 magnification for (a) unmodified keratin, (b) modified keratin, (c) UK 100%, (d) UK 90%: Chitosan 10%, (e) UK 80%: Chitosan 20%, (f) UK 70%: Chitosan 30%, (g) UK 60%: Chitosan 40%, (h) UK 50%: Chitosan 50%, (i) MK 100%, (j) MK 90%: Chitosan 10%, (k) MK 80%: Chitosan 20%, (l) MK 70%: Chitosan 30%, (m) MK 60%: Chitosan 40%, (n) MK 50%: Chitosan 50%. Figure 01 indicates that the MK structure has re-arranged upon keratin modification. Further, the SEM images of MK scaffolds appeared smoother with highly porous structures compared other scaffolds. Porosity increased with increment of chitosan.

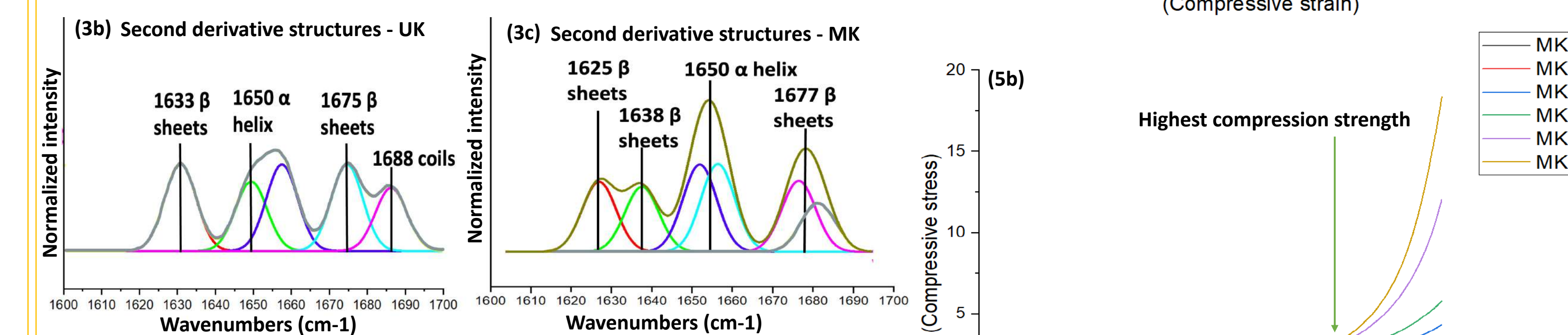
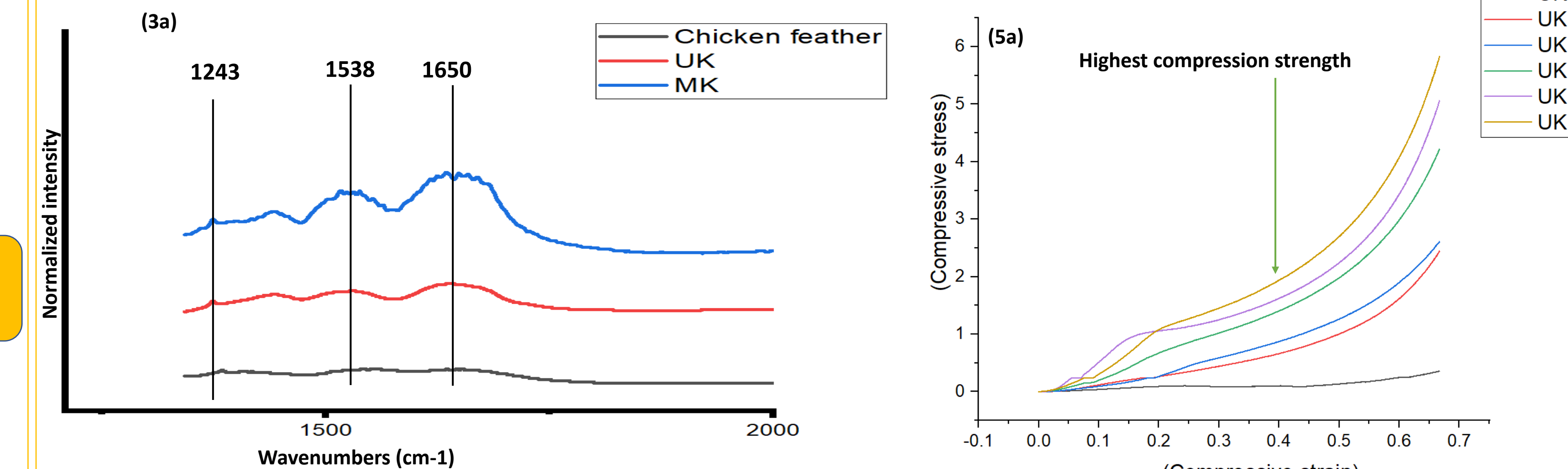


Figure 03: (3a) Absorbance intensities of Tyr ring-OH group were significantly higher for MK at 1520 cm<sup>-1</sup> and C-H aromatic groups at 3295-4200 cm<sup>-1</sup>, the peak for C-H aromatic groups at 3295-4200 cm<sup>-1</sup> has also shifted further confirming the conversion of Tyr residues of protein secondary structure into DOPA groups upon Tyrosinase modification. 3b and 3c shows the structural changes for amide I peak.

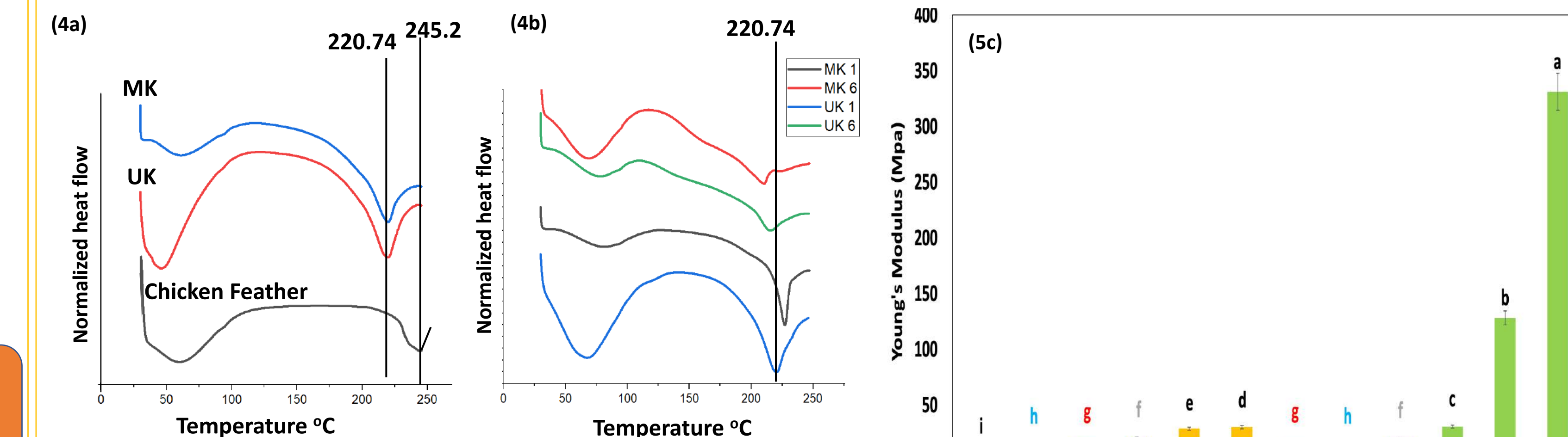


Figure 04: (4a) Endothermic peaks of DSC thermograms for chicken feather (245.2°C), UK and MK (220.74°C) suggested that the extraction slightly changed the thermal stability of keratin structure. (4b) Further, blending with chitosan also slightly changed the thermal stability of keratin: chitosan scaffolds.

Figure 05: Compressive strength obtained for UK (5a) and MK (5b) scaffolds were increased gradually with the increment of chitosan %. Young's modulus (5c) was also observed the same gradual increment showing the highest strength for MK 6 (MK 50% : Chitosan 50%) scaffolds. Letter "a,b,c,d,e,f,g and h" show the Tukey mean comparison of the scaffolds (P<0.05).

## Conclusions

- Keratin modification with addition of DOPA positively affected the surface hydrophobicity of the native keratin structure.
- Blending of modified keratin with chitosan modified the crystalline properties, increased the mechanical strength, and the amount of pores in the scaffolds. MK 50%: Chitosan 50% Scaffold was the most porous structure with highest strength.
- Biomimetic modification improved the keratin structure & fabricating scaffolds with chitosan increased the overall mechanical properties.

## References