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Mateus da Silva Simplício

Interação entre proteínas vegetais hidrolisadas e cabelo termicamente danificado por FT-IR (ATR)

Florianópolis
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Interaction between hydrolyzed vegetable proteins and heat damaged hair by FT-IR (ATR) analysis

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Resumo:

O cabelo consiste principalmente de proteínas em α-hélice conhecidas como α-queratinas, bem como proteínas associadas à queratina, responsáveis por dar ao cabelo suas propriedades características, como força e forma. Ferramentas térmicas para modelagem dos cabelos podem danificar extensivamente a cutícula do cabelo, tornando a haste do cabelo menos protegida, mais quebradiça e porosa. Proteínas hidrolisadas têm sido amplamente utilizadas em cosméticos capilares devido à sua propriedade de substantividade. Diante disso, o objetivo deste estudo foi avaliar a extensão do dano térmico capilar causado pelo tratamento térmico, assim como a avaliação da interação entre proteínas vegetais hidrolisadas e a superfície danificada do cabelo por espectroscopia no infravermelho por transformada de Fourier (FT-IR). Em geral, o dano térmico provocou a diminuição da intensidade de algumas bandas (2923, 2853, 1633 e 1043 cm^{-1}) e o desaparecimento da banda em 798 cm^{-1} em relação aos cabelos

não danificados. Além disso, a banda Amida II deslocou para números de onda mais baixos, indicando uma possível mudança na conformação da proteína. Os resultados podem indicar que as proteínas vegetais hidrolisadas são capazes de formar interações fracas (efeito de substantividade), mas não são capazes de formar ligações fortes, como ligações covalentes, na superfície do cabelo danificado pelo calor. Isso significa que elas são úteis principalmente em produtos capilares *leave-in*, e que o uso contínuo do produto é necessário para manter os benefícios cosméticos da fibra.

Palavras-chave: proteína hidrolisada, cabelo humano, danos térmicos, espectroscopia no infravermelho.

Abstract:

Hair mostly consists of α-helical proteins known as α-keratins as well as keratin-associated proteins which are responsible for giving hair its characteristic properties, such as strength and shape. Thermal styling tools may extensively damage the hair cuticle therefore turning the hair shaft less protected, more brittle, and porous. Proteins hydrolyzates have been widely used in hair care cosmetics due to its substantivity property. Considering this, the aim of this study was to evaluate the extent of the hair thermal damage caused by a hot flat iron treatment, in addition to the evaluation of the interaction between hydrolyzed vegetable proteins and the damaged hair surface by fourier transform infrared (FT-IR) spectroscopy. In general, the heat damage caused the lowering of the intensity of some bands (2923, 2853, 1633, and 1043 cm^{-1}) and the disappearance of the band at 798 cm^{-1} compared to hair not exposed to thermal damage. Also, the Amide II band shifted to lower wavenumbers indicating a change in protein conformation. Our results may indicate that the hydrolyzed vegetable proteins are able to form weak interactions (substantivity effect), but are not able to form strong bonds, such as covalent bonds on the heat damaged hair surface. This means that they are mainly

useful in leave-in hair care products, and that continued use of the product is necessary to maintain the fiber's cosmetic benefits.

Keywords: hydrolyzed protein, human hair, thermal damage, infrared spectroscopy.

1. Introduction

Human hair has 65-95% (depending on humidity) of its weight made of proteins (mainly α-keratins), while the rest is water, lipids, minerals, and others (ROBBINS, 2012; LIMA *et al.,* 2016; PIENPINIJTHAM *et al.*, 2018). Hair is structured in three concentric layers (Figure 1): the cuticle (outermost layer), the cortex, and the medulla (innermost layer). The medulla is usually not present all the way along the hair shaft and completely absent in thin hair (BARTON, 2011; ROBBINS, 2012). The cortex is the main structural component being the central core in the hair shaft. It is composed of elongated spindle-shaped keratinized cells packed tightly, parallel to the axis of the fiber (POPESCU & HÖCKER, 2007; BARTON, 2011; ROBBINS, 2012). The cells main component is α-keratin, which accounts for around 40% of the fiber's cross section (ZHOU *et al.*, 2011). These α-keratins are the component units of keratin intermediate filaments, which along with the amorphous matrix of keratin associated proteins constitute the macrofibrils, that run throughout the cortex of the hair (POPESCU & HÖCKER, 2007; RICHENA & REZENDE, 2019).

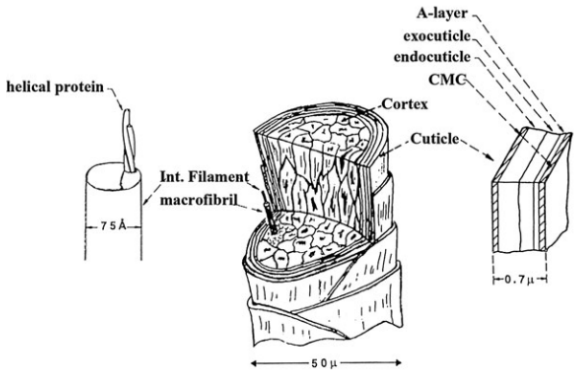

Figure 1. Hair fiber structure of the cortex and cuticle (ROBBINS, 2012)

The cuticle is a protective layer made of scale-like cells that overlap each other, giving the appearance of a tiled roof (POPESCU & HÖCKER, 2007; BARTON, 2011; ROBBINS, 2012; LOURENÇO *et al.*, 2021). Each cuticle cell is enveloped by a strongly adhesive layer known as the cell membrane complex (CMC), which is a layer rich in lipids (mainly 18-methylhexanoic acid) covalently bound to proteins (BARTON, 2011; ROBBINS, 2012; RICHENA & REZENDE, 2015). The cuticle cell is mostly an amorphous matrix with different layers of varying disulfide and isodipeptide content (POPESCU & HÖCKER, 2007; RICHENA & REZENDE, 2015). The first one being the endocuticle, which is made up of non-keratinous material, is cystine-deficient and hydrophilic; the second layer is the exocuticle and it represents two-thirds of the cuticle structure cell. The proteins here are densely cross-linked by disulphide bonds of cystine, but not as extensively as the A-layer. The third being the A-layer, which is both hydrophobic and more reticulated, due to the higher cystine content, and thus is characterized as a more biochemically stable layer, being strongly resistant to mechanical and chemical forces. Finally, the epicuticle, closer to the surface, is hydrophobic and cystine-rich. The cystine groups of the epicuticle are bound to the CMC (POPESCU & HÖCKER, 2007; BARTON, 2011; ROBBINS, 2012; RICHENA & REZENDE, 2015; ROGERS, 2019).

The cysteine present in hair keratin largely contributes to the stability of the fiber through covalently bond disulphide (-S-S-) cross-links. They are the strongest type of bonds present in hair and thus contribute greatly to the strength and stability of the hair fiber. Other weaker interactions include coulombic interactions, hydrogen bonds, van der Waals interactions and hydrophobic bonds (POPESCU & HÖCKER, 2007; BARTON, 2011).

Straightening and curling styling of hair are sources of damage to the shaft (GAMA *et al.*, 2011; FARIAS, 2019; LIMA *et al.*, 2019; BARRETO *et al.*, 2021). Many of them involve heating and may cause harm to the hair even in relatively low temperatures (LIMA *et al.*, 2019). For example, thermal damage can cause the transition in conformation of α-keratin into a β-pleated

sheet, altering the bonding structure and therefore the stability of hair (ZHOU *et al.,* 2011). The straightening effects of the heat treatment are primarily due to the breakage and reformation of hydrogen bonds in the protein structures. The rearranged bond network is reasonably stable until exposure to humid conditions or washing (WORTMANN *et al.*, 2018).

Proteins have been widely used in hair care cosmetics. They can render hair shiny, smooth and frictionless due to its substantivity property, which results from the many polar and ionic bonding sites (SECCHI 2008; GAMA *et al.,* 2011; DIAS 2015). Hydrolyzed proteins may be available at smaller molecular sizes (<1000 Da) and therefore are used in hair care formulations due to their ability to penetrate more deeply into the hair shaft. On the other hand, the bigger molecular sizes (>1000 Da) can accumulate on the cuticle conferring body and protection until the next shampooing (GAMA *et al.*, 2011; DIAS, 2015; DRAELOS, 2013).

Fourier transform infrared (FT-IR) spectroscopy is a technique based on the determination of infrared absorption due to energy resonance with vibrational motions of chemical bonds (BARTON, 2011; PIENPINIJTHAMA *et al.*, 2018). It is useful to evaluate the human hair and damage that the fiber may have suffered since it can detect changes in bond structure (PANAYIOTOU & KOKOT, 1999; LOURENÇO, 2021). Attenuated Total Reflectance sampling technique makes the sample preparation easier. The infrared light is reflected by the ATR crystal above which the sample is placed. Each reflection will generate a wave called “evanescent wave” that penetrates only a short distance into the sample. Therefore, it is classified as a surface sensitive technique, which means it makes possible to obtain information about near surface bonds in the cuticle and periphery of the cortex (BARTON, 2011; PIENPINIJTHAMA *et al.*, 2018; LOURENÇO, 2021).

Considering this, the aim of this study was to evaluate the changes in the protein bond structure of thermally damaged hair as well as the possible interaction between hydrolyzed proteins (used in hair care cosmetics) and the damaged hair shaft, using FT-IR analysis.

2. Material and methods

2.1 *MATERIAL*

Caucasian dark brown untreated hair tresses (weighing around 1 g and 20 cm long each) were voluntarily donated with informed consent by a female volunteer. The hair tresses were collected by the authors after the study was approved by the Ethics Committee of the Federal University of Santa Catarina, Brazil (CAAE: 53005521.5.0000.0121).

The vegetable protein hydrolysates: Gluadin® Kera-P LM (Hydrolyzed Vegetable Protein ~1500 Da) and Gluadin® WLM Benz (Hydrolyzed Wheat Protein <1000 Da) were provided by BASF (São Paulo, SP, Brazil).

2.2 *METHODS*

2.2.1 Sample pre-wash

Before the thermal treatment, the hair tresses were washed to remove any residues from the hair. First, the samples were wet in warm distilled water (40°C) and washed with a 10% (w/w) dispersion of sodium lauryl ether sulfate (SLES) through gentle massage between the fingers, moving from root to end. Then, they were rinsed with warm distilled water and hung to dry in ambient conditions. From this step, the control hair was obtained.

2.2.2 Thermal treatment

A pre-washed hair tress was selected and submitted to a thermal treatment. The treatment consisted of 70 slow passes (10 seconds each) through the hair tress (20 cm) using a hot flat iron at around 208°C (the temperature was checked with an infrared thermometer). From this step, the heat damaged hair was obtained.

2.2.3 Vegetable protein hydrolysates treatment

The heat damaged hair was submitted to two distinct treatments with vegetable protein hydrolysates of different molecular sizes. For each treatment, a heat damaged hair tress was submerged in 50 mL of a 2% (w/w) dispersion of a protein hydrolysate (Table 1) for 10 min: hydrolyzed wheat protein (molecular size <1,000 Da) or hydrolyzed vegetable protein (molecular size = 1,500 Da). After 10 min had passed, the treated hair tresses were either rinsed with water or washed with SLES 10% (Figure 8). From this step, the two protein-treated hair samples were obtained.

Table 1. Hydrolyzed proteins utilized in this study.

Name INCI	Producer description	Molecular size (Da)	Concentration*	Solution pH
Hydrolyzed vegetable protein	Low molecular weight peptides that penetrate deeply penetrate the hair shaft	1,500	2%	4,2
Hydrolyzed wheat protein	Low molecular weight peptides good for repairing benefits in cosmetic formulations	<1,000	2%	4,2

*Concentration suggested by the manufacturer for the final product.

2.2.4 *FTIR-ATR analysis*

Some fibers of each hair sample (control, heat damaged and two protein-treated hair) were selected and cut into snippets (about 2.0 mm in length) from which the IR spectra were obtained. The IR spectra of the hair samples were recorded with 16 scans in the 500 to 4000 cm^{-1} range with 4 cm^{-1} resolution, using the PerkinElmer Frontier MIR single-range FTIR spectrometer. All spectra were baseline corrected using Spectragryph v. 1.2.16 software.

3. RESULTS AND DISCUSSION

3.1 *Untreated hair (control)*

Figure 2 shows the IR spectra of control hair sample (not damaged by heat) where characteristic bands from hair can be identified. The results seem to be in agreement with the literature (ZHOU *et al.,* 2011; PIENPINIJTHAMA *et al.,* 2018; LIMA *et al.*, 2019; BARTON 2011; RICHENA & REZENDE, 2015).

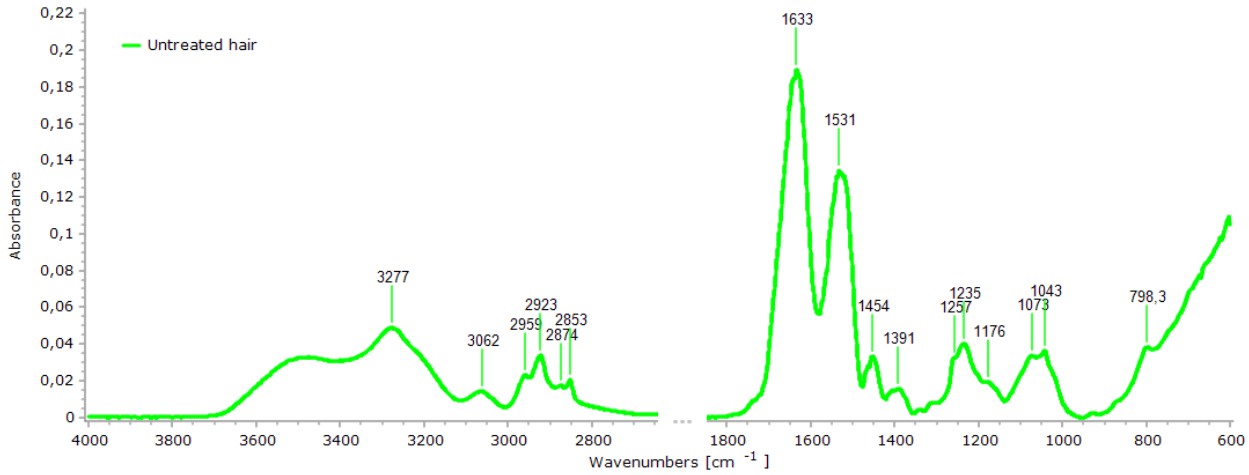

Figure 2. FTIR-ATR spectra of control untreated hair (not damaged by heat).

The region between 1750 and 750 cm^{-1} contains the major amide and C-H deformation bands, which are characteristic of the peptide linkage of the proteins (PANAYIOTOU & KOKOT, 1999). The predominant bands 1633, 1531 and 1235 cm^{-1} arise from peptide bonds and are associated with the Amide I, Amide II and Amide III vibrations, respectively (PANAYIOTOU & KOKOT, 1999; BARTON, 2011, PIENPINIJTHAM *et al.*, 2018). The absorption of the Amide I

(1690-1600 cm^{-1}) band at 1633 cm^{-1} is assigned to stretching vibrations of the C=O bond of the amide in β-pleated conformation (JACKSON & MANTSCH, 1995; BARTON, 2011) and a small contribution from N-H bond bending (scissoring); the amide II (1575-1480cm^{-1}) band at 1518 cm^{-1} is assigned mainly to bending vibrations of the N-H bond and some stretching vibrations of the C-N bond (RICHENA & REZENDE, 2015; PIENPINIJTHAM *et al.,* 2018). The Amide III (1320-1210cm^{-1}) band at 1235 cm^{-1} is assigned to N-H bending (twisting), C-N stretching, with contributions of O=C-N bending vibration (BARTON, 2011; RICHENA & REZENDE, 2015).

Figure 3 shows the band at 3277 cm^{-1}, assigned as the Amide A, which arises from N-H bond stretching and is sensitive to hydrogen bonding, essential for the secondary structure of the hair protein (KIM & PARK, 2013). The absorption bands between 2960-2850 cm^{-1} are assigned to the C-H stretching and bending modes of CH_3 and CH_2 related mainly to proteins but with a minor contribution of lipids in the hair (ZHOU *et al.*, 2011; PIENPINIJTHAMA *et al.*, 2018; LIMA *et al.*, 2019).

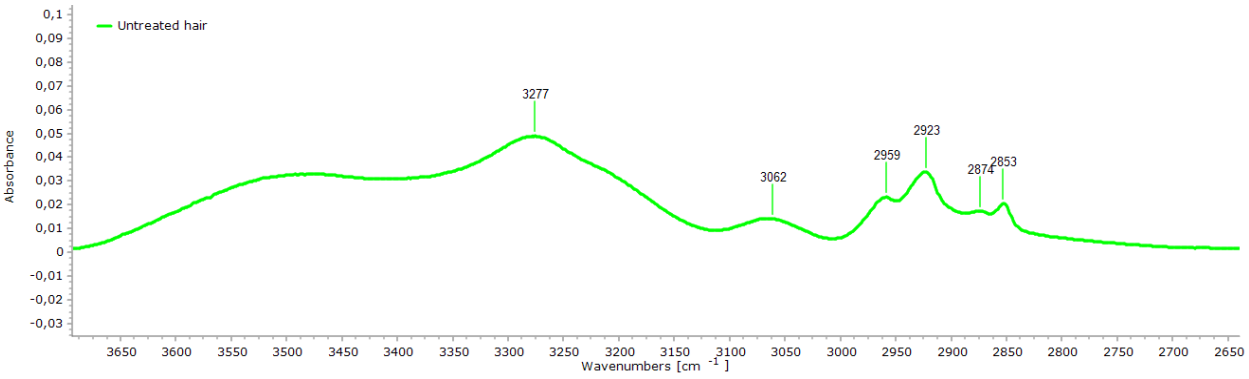

Figure 3. Detailed view (3500-2800 cm^{-1}) of the FT-IR spectra of untreated hair (not damaged by heat) .

In Figure 4, at 1739 cm^{-1}, mostly obscured by the Amide I band appears a small band assigned to the C=O stretch (PANAYIOTOU & KOKOT, 1999; BARTON, 2011). The next series of absorptions obtained in the spectra are attributed to C-H bending vibrations of CH_2 (1450 cm^{-1}) and CH_3 (1391 cm^{-1}) groups originating from the various amino acid side chains (BARTON, 2011; PIENPINIJTHAMA *et al.,* 2018).

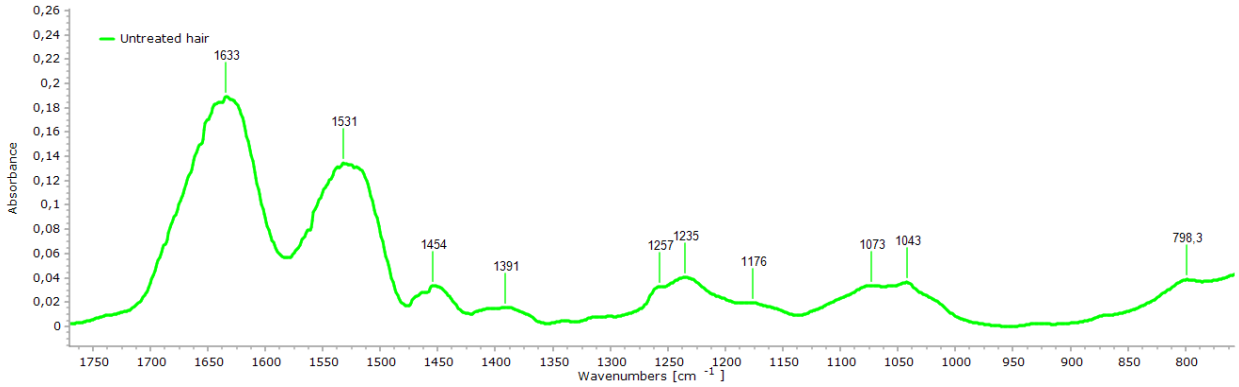

Figure 4. Detailed view (1800-600 cm^{-1}) of the FTIR spectra of untreated hair ((not damaged by heat).

Between 1200-1000 cm^{-1} are the IR absorptions associated with the oxidation of the disulfide bond, often related to oxidative damage due prolonged sun exposure and swimming in chlorinated water (BARTON, 2011; RICHENA & REZENDE, 2015). In this range, the bands of cysteic acid (SO_3^-) and the cystine monoxide (S=O) are observed at 1043 and 1073 cm^{-1}, respectively (BARTON, 2011).

3.2 *Heat damaged hair*

The IR spectra of the heat damaged hair sample (Figure 5) presented minimal differences compared to the untreated hair sample, in accordance with the literature (SANTOS, 2017; LIMA *et al.,* 2019). In the first region of the spectra (3800-2800 cm^{-1}) the heat damage caused a lowering of the intensity of the bands. Most notably the bands at 2923 and 2853 cm^{-1} were lowered, which are attributed to asymmetric and symmetric stretching vibrations of C-H bonds of CH_2 groups (RICHENA & REZENDE, 2017; PIENPINIJTHAMA *et al.*, 2018). This may suggest a loss of protein and lipids due to the cuticle damage by the thermal treatment (LIMA *et al.*, 2019). In the second region between 1800-800 cm^{-1}, the band related to Amide I (1633 cm^{-1}) presents a lower intensity in the heat damaged sample than in the untreated sample. The Amide II band correlated to the C–N bond stretch and N–H bond in-plane-bending of the β-pleated sheet conformation, appears to shift from 1531 to 1516 cm^{-1} in the untreated and thermally damaged samples, respectively. According to a second derivative analysis of the FT-IR spectra, Zhou et al. (2011) observed that the Amide II band has contributions from both α-helical structure and β-sheet, the first at ca. 1548 cm^{-1} and the second ca. 1516 cm^{-1}. Thus this band shift to lower wavenumbers could be indicative of changes in hair keratin conformation from α-helical to β-sheet.

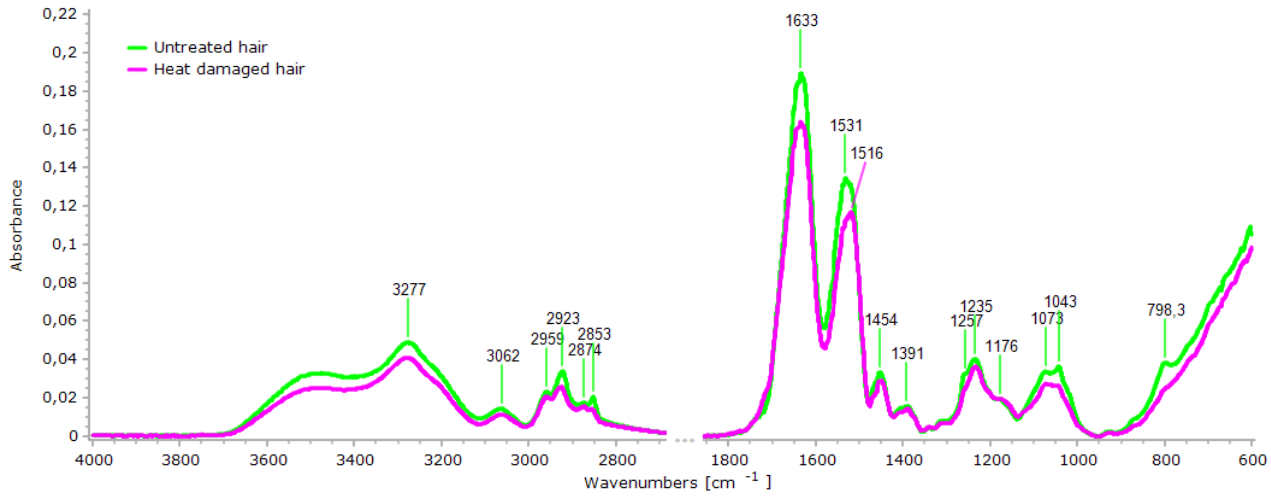

Figure 5. Comparison between FT-IR spectra of untreated hair (control) and heat-damaged hair.

Additionally, a more pronounced band shoulder peak (related to Amide III bands 1320-1210 cm^{-1}) at 1257 cm^{-1} was noted in the unheated hair relative to the thermally treated hair (Figure 5). Differently, Lima et al. (2019) observed the appearance of the Amide III band at 1259 cm^{-1} for the thermally treated hair, related to β-sheet conformation. Next to the Amide III region is the cystine oxidation products region (1200-1000 cm^{-1}) in which it can be observed the flattening of the band at 1043 cm^{-1} in the thermally treated hair. It is possible that the cuticle may have been extensively damaged due to the heat and some parts may have detached and fallen, including many of the regions containing these oxidation products.

Lastly, another difference between the spectra of the heated and not heated hair samples comes from a band at around 798 cm^{-1}. This band is present in the undamaged hair is assigned to out-of-plane deformation N-H bond in NH_2 (PANAYIOTOU, 1999; LIMA *et al.*, 2019).

3.3 *Hydrolyzed protein treated hair*

Figure 6 shows the FTIR-ATR spectra of the heat-damaged hair with and without the protein-treatment (1500 Da) plus water rinsing.

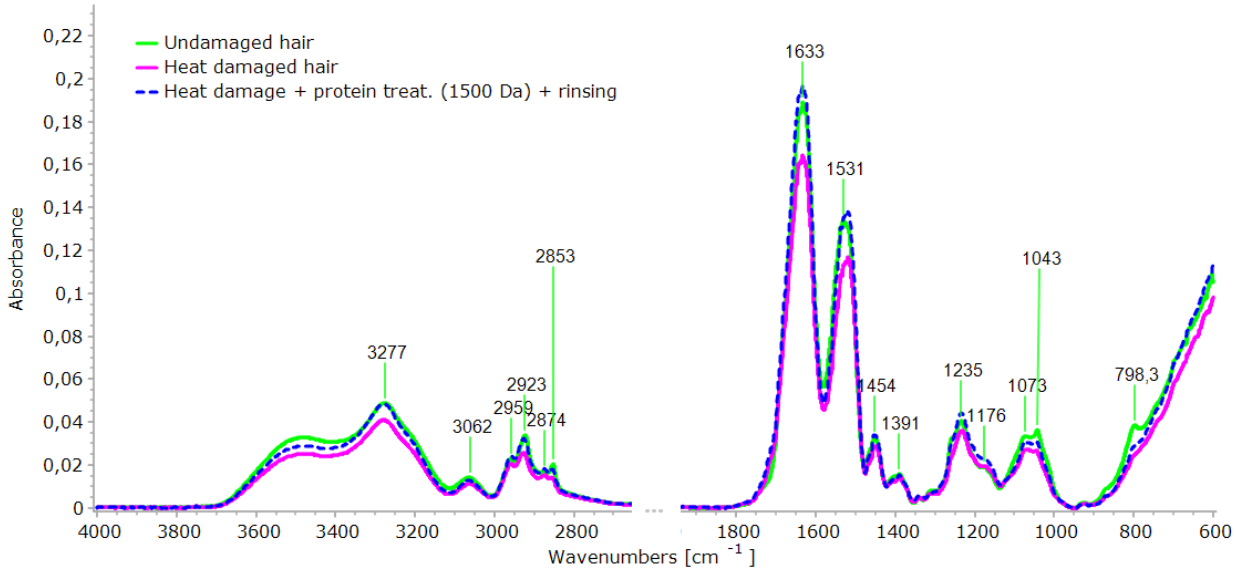

Figure 6. FT-IR spectra of three hair samples: untreated hair (control), heat damaged, and protein treated (1500 Da) plus rinsing.

The main differences between the spectra of the heat-damaged hair with and without the protein treatment (<1000 and 1500 Da) are in the intensity of the absorption bands. The sample that received the protein treatment (1500 Da) is closest in band intensity to the untreated reference, except the bands at 1043 cm^{-1} and 798 cm^{-1} (Figure 6). An hypothesis for this could be the hydrolyzed vegetable protein adsorbs or deposits itself in the gaps present on the cuticle, which were caused by the heat treatment. This may be reinforced because the bands at 1043 and 799 cm^{-1} (which are related to the hair's natural keratin) were not observed in the protein

treated hair. The fact that the hair was rinsed before the FT-IR analysis may suggest the presence of interactions between the hydrolyzed protein and the hair. However it's hard to make assumptions on FTIR-ATR data alone, and other studies may be needed to fully explain it. The same happens with the hydrolyzed wheat protein (<1000 Da) as shown in Figure 7.

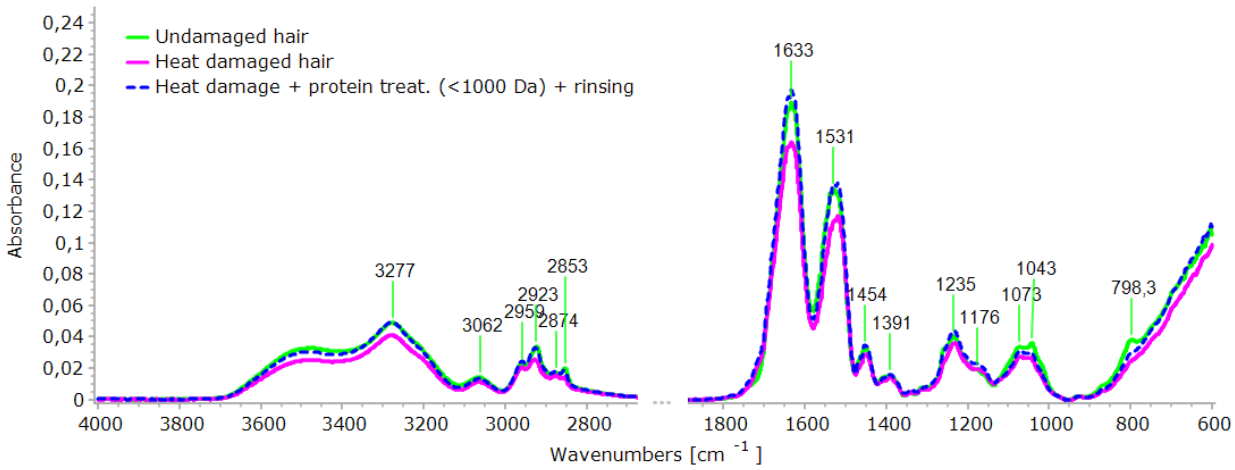

Figure 7. FT-IR spectra of three hair samples: untreated hair (control), thermally treated, and protein treated (<1000 Da) plus water rinsing.

Finally, Figure 8 shows a comparison between the thermally damaged hair and the protein treated (<1500 Da) hair after washing with SLES 10%. Unlike the water-rinsed hair, the detergent seemed to remove all the protein adsorbed on the hair surface. This may suggest that the proteins weren't able to form strong interactions with the hair keratin (such as covalent bonds) and therefore were washed away completely, as indicated by the absorption bands returning to almost exactly the shape line and maxima before the protein treatment. Zhou *et al* (2011) observed that heat treatments such as heat irons at 232°C may cause the cuticle to build cracks, holes and micropores, which could theoretically help the proteins penetrate deeper into

the shaft, but as they weren't able to form strong bonds with the hair keratin, the washing with SLES 10% was strong enough to remove it completely.

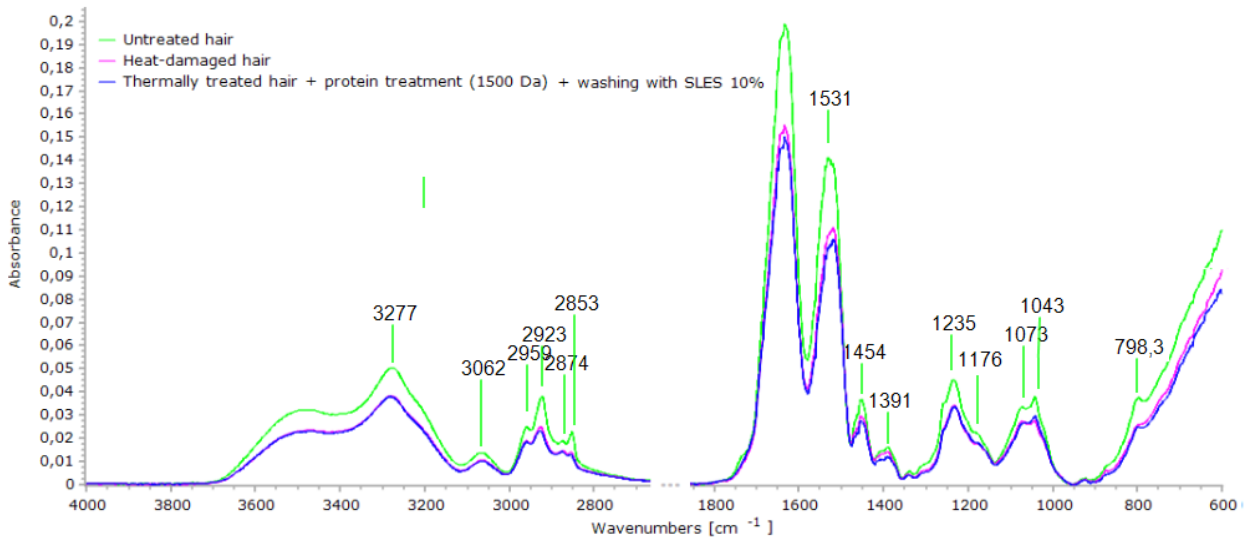

Figure 8. FT-IR spectra of two hair samples: thermally treated and protein treated (<1500 Da) plus SLES washing.

4. CONCLUSION

Our results indicate that the hydrolyzed vegetable proteins are able to form weak interactions (substantivity effect), but are not able to form strong bonds, such as covalent bonds on the heat damaged hair surface. This means that hair care cosmetics containing these protein hydrolyzates are useful as hair conditioners specially in leave-in formulations. Therefore this

may indicate that the benefits of these hydrolyzed proteins are not permanent, requiring the continuous use of the product to maintain the positive effects on the hair fiber. However, additional experiments are needed to more confidently assess the repair benefits of the hydrolyzed proteins as well as the penetrating capabilities of these proteins into the cortex. For example, scanning electron microscopy could aid in imaging the interaction between the proteins and the damaged cuticle.

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