

Research Article

The effect of additives and mechanical agitation in surface modification of acrylic fibres by cutinase and esterase

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The surface of an acrylic fibre containing about 7% of vinyl acetate was modified using *Fusarium solani pisi* cutinase and a commercial esterase, Texazym PES. The effect of acrylic solvents and stabilising polyols on cutinase operational stability was studied. The half-life time of cutinase increased by 3.5-fold with the addition of 15% *N,N*-dimethylacetamide (DMA) and by 3-fold with 1 M glycerol. The impact of additives and mechanical agitation in the protein adsorption and in the hydrolysis of vinyl acetate from acrylic fabric was investigated. The hydroxyl groups produced on the surface of the fibre were able to react specifically with Remazol Brilliant Blue R (cotton reactive dye) and to increase the colour of the acrylic-treated fabric. The best staining level was obtained with a high level of mechanical agitation and with the addition of 1% DMA. Under these conditions, the raise in the acrylic fabric colour depth was 30% for cutinase and 25% for Texazym. The crystallinity degree, determined by X-ray diffraction, was not significantly changed between control samples and samples treated with cutinase. The results showed that the outcome of the application of these enzymes depends closely on the reaction media conditions.

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1 Introduction

The interest in protein/reaction engineering in biotechnology, for the design of enzymatic modifications, has been increasing steadily as the number of successful applications in industry and the understanding of enzyme properties expand [1]. In textiles, the surface modification is considered an important path in improving the quality of natural and synthetic fibres and their processing properties [2]. The major advantages of enzymes in polymer modification compared to the chemical methods are

milder reaction conditions and easier control, environmental friendlier process, and specific non-destructive transformations on polymer surfaces [3].

Acrylic fibres (including modacrylic) cover a broad range of products, very diversified in composition and in end-use. The major reason for this is the ease of copolymerisation of polyacrylonitrile (PAN) with other monomers [4]. In spite of the chemical and physical resistance, the compact paracrystalline structure of PAN and high glass transition temperatures render to the fibres some undesirable properties that make processing and finishing methods difficult. A group of enzymes involved in the nitrile metabolism can be used to modify PAN polymers, converting the pendent nitrile groups into the corresponding amides and rendering them more hydrophilic [2, 5]. Other possible substrates in the acrylic fibres for new enzymes are the co-monomers, like vinyl acetate, present in many PAN products [3].

In a previous study, it was reported that cutinase could be used to modify the acrylic surface [6]. After a long enzymatic treatment, the hydrolysis of ester bonds from the

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Abbreviations: CI, colour index; DMA, *N,N*-dimethylacetamide; EG, ethylene glycol; K/S, Kubelka-Munk relationship (K, adsorption coefficient; S, scattering coefficient); PAN, polyacrylonitrile; p-NP, *p*-nitrophenol; p-NPB, *p*-NB butyrate; r, radius

co-monomer vinyl acetate produced acetic acid and hydroxyl groups on the surface of the fibre that could be detected by reactive staining. *Fusarium solani pisi* cutinase (EC 3.1.1.74) is an extracellular enzyme able to degrade cutin, the lipid-polyester natural coating of plants, thus rendering phytopathogenicity to the fungus from which it originates. It is a small ellipsoid protein (~22 kDa, $45 \times 30 \times 30 \text{ \AA}$) that belongs to the class of serine esterases and to the superfamily of α/β -hydrolases [7]. The versatility of cutinase in respect to possible soluble, insoluble and emulsified substrates makes it an attractive biocatalyst for a vast range of applications.

The major purpose of this work was to reduce the treatment time of acrylic fabric with cutinase and to improve its catalysis efficiency by means of reaction media manipulation. The influence of known acrylic solvents and the influence of known stabilisers on cutinase operational stability were investigated. In addition, the impact of mechanical agitation and additives on vinyl acetate enzymatic hydrolysis by cutinase and by a commercial esterase are reported.

2 Materials and methods

2.1 Reagents

The enzymes used in this study were a cutinase (EC 3.1.1.74) from *Fusarium solani pisi*, and a commercial esterase Texazym PES, from inoTEX Ltd. (Dvur Kralove nad Labem, Czech Republic). The recombinant wild-type cutinase was overexpressed in *Saccharomyces cerevisiae* SU50 strain and supplied as culture medium, with a purification degree of 50–70% with respect to total protein [6, 8]. It was a generous gift from Centro de Engenharia Biológica (Instituto Superior Técnico, Lisbon, Portugal).

Acrylic taffeta fabric used was a copolymer of polyacrylonitrile and 7% vinyl acetate, with 82 g/m^2 and 36/36 ends/picks per cm, supplied by Fisipec (Lavrado Portugal).

The reactive dye Remazol Brilliant Blue R, CI 61200, was from Sigma. All other reagents were laboratory grade reagents from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Esterase activity assay

Esterase activity was determined following the product release (*p*-nitrophenol, *p*-NP) through the increase in the absorbance at 400 nm. The activity assay conditions for cutinase were described previously [9, 10]. The esterase activity of Texazym PES was also determined using *p*-NB butyrate (*p*-NPB) as substrate, but using slightly different conditions that were optimised for this enzyme. The enzymatic reaction was started with the addition of 0.1 mL $10 \text{ mg}\cdot\text{L}^{-1}$ Texazym PES to a final volume of 2 mL of 50 mM phosphate buffer pH 8, containing 75 \mu M *p*-NPB and 5% ethanol. The mixture was incubated for 1.5 min in a wa-

ter bath, at 35°C. The hydrolysis was stopped by adding 2 mL acetone. All the assays were performed in triplicate. Standard solutions of *p*-NP were used to obtain the calibration curve.

2.3 Stability of cutinase

The operational stability of cutinase was investigated in the presence of two organic solvents and several polyols. The enzyme was incubated at a final concentration of $1 \text{ mg}\cdot\text{L}^{-1}$ in 50 mM phosphate buffer pH 8, containing 0.05% sodium azide. The concentrations of *N,N*-dimethylacetamide (DMA) or DMF used in this study were 7.5%, 15%, 25% and 50%. The final concentration of glycerol, sorbitol, xylitol and ethylene glycol (EG) used was 1 M. For each assay, the control was identically prepared except that buffer substituted the enzyme. The incubation took place in a water bath at 35°C with an orbital agitation of 72 rpm until the drop in esterase activity was above 50%.

2.4 Enzymatic treatment of acrylic fabric

All samples of acrylic fabric used were previously washed to remove impurities. The washing consisted in several steps, all performed in a laboratory scale machine, the Rotawash MKIII (vertical agitation simulating European washing machines, from SDL International Ltd.), at 60°C and 20 rpm. The fabric was washed twice for 30 min with $0.1 \text{ g}\cdot\text{L}^{-1}$ Lutensol AT25 (non-ionic detergent) and left for 10 min under running tap water. The fabric was then washed once for 30 min with $2 \text{ g}\cdot\text{L}^{-1}$ sodium carbonate and left for another 10 min under running tap water. Finally, the fabric was washed three times in distilled water for 20 min each and was left to dry at room temperature.

Two sets of experiments were carried out for each enzyme taking into account the degree of mechanical agitation. For both sets, the treatment of acrylic fabric was performed in stainless steel pots of 450 mL capacity in a Rotawash machine, in the case of cutinase, and in Washtec-P 05/99A (vertical agitation simulating European washing machines, from Roaches International Ltd.), in the case of Texazym. All the treatments were performed in 50 mM phosphate buffer pH 8, at 30°C and 20 rpm, for 3 h. To increase the mechanical agitation, stainless steel discs ($19.2 \pm 0.2 \text{ g}$, $32 \times 3 \text{ mm}$) were added to the reaction mixture. In the cutinase treatment, samples of acrylic fabric, with an average weight of $1.57 \pm 0.01 \text{ g}$, were incubated with $9 \pm 2 \text{ U}$ ($\mu\text{mol}_{\text{p-NP}}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$) of cutinase, in a final volume of 100 mL. Five different media were tested: no additives, 1% and 15% DMA, 1 M glycerol and 1% DMA + 1 M glycerol. In a second set of assays, these conditions were repeated and four stainless steel discs were added to each assay. In the treatment with Texazym PES, samples of acrylic fabric, with an average weight of $3.05 \pm 0.02 \text{ g}$, were incubated with $3.4 \pm 0.5 \text{ U}$ ($\mu\text{mol}_{\text{p-NP}}\cdot\text{min}^{-1}\cdot\text{mL}^{-1}$) of the enzyme, in a final volume of

200 mL. The reaction media were the same as described for cutinase. The number of discs added was raised to nine per assay. For each reaction media, a control was run in parallel in which buffer substituted the enzyme.

After enzymatic treatment, all fabric samples were washed twice in $2 \text{ g}\cdot\text{L}^{-1}$ sodium carbonate for 30 min, once in $0.25 \text{ g}\cdot\text{L}^{-1}$ Lutensol AT25 for 30 min and three times in distilled water for 15 min, in the Rotawash/Washtec-P at 70°C .

2.5 Quantification of total protein concentration

Total protein in solution was quantified following Bradford methodology [11], using BSA as standard. All samples were measured in triplicate.

2.6 Determination of acetic acid concentration in the bath solutions

Detection of acetic acid in reaction media was performed with a kit from Boehringer Mannheim/R-Biopharm (cat. no.10148261035; Darmstadt, Germany). Protein was previously precipitated using perchloric acid according to the instructions supplied.

2.7 Acrylic fabric staining with a reactive dye

After enzymatic treatment, samples were competitively stained in duplicate with 2% (of weight of fabric) Remazol Brilliant Blue R, CI 61200. The staining bath contained in a $10 \text{ g}\cdot\text{L}^{-1}$ sodium carbonate solution. The staining was performed at 70° and 80°C , in a lab dyeing machine (AHIBA Spectradye, from Datacolor International), for 90 min at 20 rpm. The total fabric average weight per staining assay was $2.87 \pm 0.02 \text{ g}$ and $2.18 \pm 0.09 \text{ g}$ for samples treated with cutinase and Texazym PES, respectively.

After staining, all samples were washed once with $0.25 \text{ g}\cdot\text{L}^{-1}$ Lutensol AT25 and several times with water at 70°C in Rotawash, until no more dye could be detected in the solution. The colour measurements were carried out with a reflectance spectrophotometer with a standard illuminant D65 (Spectraflash 600 Plus, from Datacolor International). The colour strength was evaluated as K/S at maximum absorption wavelength (595 nm). The ratio between absorption (K) and scattering (S) is related to reflectance data by applying Kubelka-Munk's law at each wavelength, and it is proportional to dye concentration [12].

2.8 X-ray diffraction

The X-ray diffraction patterns were obtained for the acrylic fabric samples treated with cutinase without and with stainless steel discs (the later for the samples treated in the presence of the additives). The X-ray diffraction

experiments were performed using a Philips PW1710 apparatus, with Cu Ka radiation and operating at a 40 kV voltage and 30 mA current. The patterns were continuously recorded in the diffraction angular range 2θ from 5° to 40° , with a step size of 0.02° at 0.6°min^{-1} .

The values of crystallinity index (*CrI*) were obtained according to the method of El-Zaher [13].

3 Results and discussion

3.1 Operational stability of cutinase

From the biotechnological point of view, both storage and operational stabilities greatly influence the usefulness of enzyme-based products [14]. Operational stability, defined as the persistence of enzyme activity under the conditions of use, was studied for cutinase before applying the enzyme to acrylic fabric treatment. The media conditions, such as buffer, pH and temperature were chosen based on preliminary studies performed in the laboratory, using the esterase activity determination methodology described earlier. The conditions chosen, which maximise the hydrolysis of p-NPB by cutinase, were phosphate buffer pH 8 and temperatures between 30° and 40°C . These preliminary results were in agreement with those described [15–17]. Under the reported conditions, the specific activities of cutinase and Texazym PES were 253 ± 51 and $11 \pm 1 \text{ U}\cdot\text{mg}^{-1}$, respectively.

The half-life times obtained for cutinase, incubated under different conditions, are shown in Table 1, as a measure of operational stability. The values attained suggest that cutinase tolerates well the two organic solvents tested if their concentration remains below 15%. The stability of cutinase was improved with 15% DMA, with the half-life time 3.5-times higher than that of the control. Concentrations of DMA and DMF above 15% drastically reduced the half-life time. Cutinase is one among other enzymes reported in literature that exhibits an increase in its stability as well as in its maximal activity (results not shown), in the presence of low concentrations of organic co-solvents [14, 18–20].

From the polyols studied, glycerol and sorbitol were the only ones improving the stability of cutinase. With glycerol, the half-life time increased by threefold. Xylitol, EG and PEG had the opposite effect, with a half-life time reduction of above 30%. The use of some low molecular weight compounds in solution has been found to stabilise native conformations of globular proteins, when added at high concentrations ($\geq 1 \text{ M}$) [21]. A common feature between these stabilisers is that they are preferentially excluded from the surface of native proteins or, in other words, proteins are preferentially hydrated, which, in the case of cutinase, induces protein stabilisation [21, 22].

The values of half-life time were calculated whenever the experimental data was adequately fitted with a first

Table 1. Influence of two organic solvents and several polyols on operational stability of cutinase. The cutinase was incubated at 35°C, pH 8, in a water bath with agitation, under different media conditions. The half-life time was calculated, whenever possible, as $\ln 2/k$, from the first order exponential decay fit of data ($a/a_0 = Ae^{-kt}$)

Assay conditions		Half-life time (days)
No additives		45 ± 7
DMF	7.5%	45 ± 4
	15%	46 ± 7
	25% ^{a)}	0.6
	50% ^{a)}	0.01
DMA	7.5%	48 ± 2
	15%	159 ± 24
	25% ^{a)}	4
	50% ^{a)}	0.4
Polyols	Glycerol ^{a)}	134
	Sorbitol ^{a)}	113
	Xylitol ^{a)}	31
	EG	29 ± 8
	PEG	23 ± 8

a) The half-life time was obtained from the second order exponential decay fitting curve.

order exponential decay, using OriginPro 7.5 (Origin Lab Corporation, USA). This was the case for the assays where cutinase was incubated with 7.5% and 15% of both organic solvents and with EG and PEG. The half-life times for cutinase incubated with glycerol, sorbitol, xylitol, and both organic solvents at 25% and 50% (v/v) were obtained from the second order exponential decay fitting data. Many protein deactivation models are not first order [14, 23–26]; in fact, the model of activity decay of cutinase seems to depend on the pH of the solution. As already reported, cutinase displayed first order exponential decay at

pH 9.2, while at pH 4.5 its deactivation was undoubtedly non-first order [17, 26].

3.2 Enzymatic modification of acrylic surface

The modification of a solid substrate, like acrylic fibres, with enzymes constitutes a heterogeneous biocatalysis, since enzyme and substrate are in different phases. Therefore, it is necessary to consider two general steps to make the catalysed reaction occur: the physical adsorption of cutinase/Texazym to acrylic fibre surface and the formation of the enzyme/substrate complex and consequent hydrolysis of the ester bond between vinyl acetate and the backbone of the polymer chain.

During the 3 h of treatment, aliquots were taken at different time intervals to follow the protein adsorption. In Fig. 1, the total protein is plotted for the cutinase treatment with different amounts of DMA, under low and high mechanical agitation. These conditions were chosen as an example. It is clear from the results that there is no significant protein adsorption under the conditions tested, taking into account the experimental error. When the discs are present, the greater mechanical agitation did not affect the adsorption behaviour of this enzyme into acrylic fibre. In addition, it was not possible to see a clear effect of the organic solvent on protein adsorption. The lack of significant adsorption was also verified in the acrylic treatment with Texazym PES for both degrees of mechanical agitation.

These results were not surprising since polyacrylonitrile polymers are polar materials and, for that, they are known low-protein-adsorbing polymers, often used to produce inert membranes and supports in bioprocessing technology [27].

In theory, if the hydrolysis of vinyl acetate happens on the surface of the surface, according to the 'electrostatic

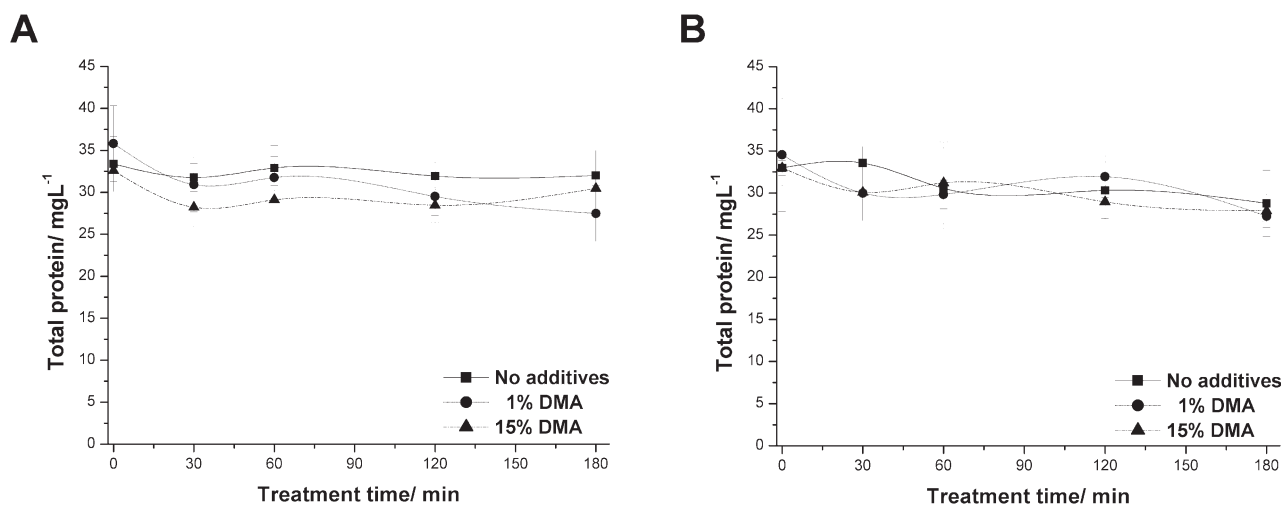


Figure 1. Total protein concentration in bath treatment solutions during the 3-h treatment of acrylic with 9 U·mL⁻¹ of cutinase and different amounts of DMA, in absence (A) and in presence (B) of stainless steel discs.

catapult model' [28], at pH 8, cutinase should release the product, acetate anion. It was not possible to detect acetic acid in the aliquots collected during the treatment of acrylic with both enzymes. The detection limit, according to the kit suppliers, is 1.5 mg·L⁻¹ acetic acid. The average acrylic weight used was 15.5 g·L⁻¹ of treatment solution. Thus, less than 0.14% of the total available vinyl acetate was hydrolysed (in average, 7% of 15.5 g·L⁻¹). According to the model proposed by Warner and colleagues [4], acrylic polymers are organised in fibres of strong laterally bound chains. Assuming that the enzyme cannot penetrate inside the fibres, the area/volume of these fibres determines the vinyl acetate available for enzyme attack. Relating the area/volume (2/r) between a single polymer chain (6 Å) (maximal accessibility), and acrylic fibres (diameter: 100–1000 Å) (<<100% accessibility), the acetic acid concentration produced would be between 6.5 and 65 mg·L⁻¹.

Factors like the use of textile fabric instead of free fibres and the lower efficiency of enzymatic catalysis in a heterogeneous system could explain the failure to detect acetic acid by this method.

A methodology was developed [6] to measure the hydroxyl groups that result from the enzymatic hydrolysis of the ester linkage of vinyl acetate and that remain attached to the polymer backbone. The basic principle is the specific reaction between a vinylsulphonic group from a reactive dye, in this case Remazol Brilliant Blue R, and the hydroxyl group at the fibre surface. The specificity of the method is increased by the fact that this kind of reactive dye was designed for cellulose fibre dyeing, thus it has low affinity for synthetic fibres. The sensitivity is also very high due to the large molar absorptivities of dye molecules.

A higher level of mechanical agitation was of crucial importance in the acrylic fabric treatment with both enzymes. Without the addition of stainless steel discs, it was not possible to measure any difference in K/S between treated samples and controls. The relative increase in K/S, obtained for the set of experiments where the discs were used, is represented in Fig. 2. Treating acrylic fabric, either with cutinase or with a commercial esterase, only led to the formation of hydroxyl groups when the stainless steel discs were introduced into the incubation vial. The experiments were performed in lab machines reproducing the vertical agitation of European washing machines, where the mechanical work involves the fibre-fibre and fibre-metal friction as well-beating effects. The introduction of metal discs increases the beating effects and the fibre-metal friction [29]. The finishing and washing effects produced by cellulases were also highly dependent on strong mechanical work delivered to cotton fabrics [30, 31]. Heterogeneous catalysis implies adsorption of the enzyme, which is in itself a complex process depending, among other issues, on transport toward the surface by convection and diffusion. Accordingly, it is likely that an increase in mechanical agitation is helpful for the outcome of acrylic biotransformation, increasing the accessibility. In addition, in a comparable way to cellulose, the increased friction could have a micro-pilling effect on the acrylic fabric, therefore, increasing the surface area available for enzymatic attack.

DMA is a known solvent of PAN and it is commonly used in acrylic fibre industrial production. Its plasticiser function disturbs the regular structure of the polymer, reducing the magnitude of inter-chain bonding, which should aid the penetration of the enzyme, improving its action on the fibre. The acrylic fabric sample treated both

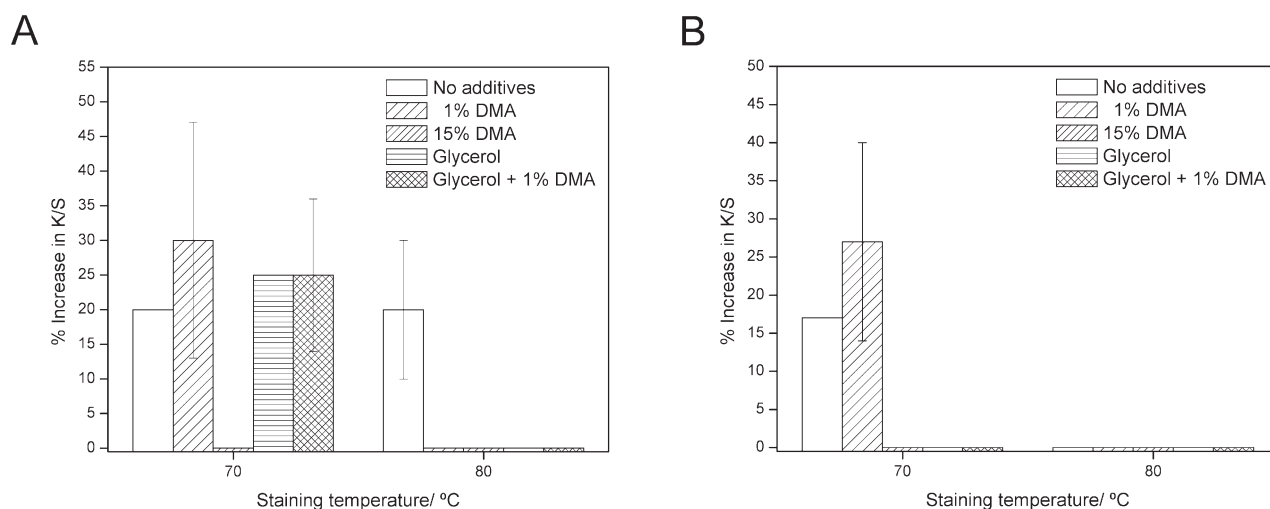


Figure 2. Percentage of increase in K/S for acrylic samples treated with cutinase (A) and with a commercial esterase (B). To increase the mechanical agitation, stainless steel discs were added to all the assays plotted. After enzymatic treatment, samples were washed to remove any protein adsorbed to the fabric. Samples were competitively stained at 70° and 80°C, and the relative K/S was calculated as $\frac{K/S_{enzyme} - K/S_{control}}{K/S_{control}}$. The controls were identically prepared for each assay conditions, except that buffer substituted the enzyme.

with cutinase and with Texazym PES in the presence of 15% DMA did not show any difference in respect to controls (Fig. 2). The increased accessibility, as the consequence of swelling of fibres by the organic solvent, does not therefore render an enhancement in cutinase catalysis on acrylic surface. If the observed increase in cutinase stability is due to the preferential hydrating phenomena induced by DMA, most probably the interactions between enzyme and polymer are not sufficiently strong to displace the tightly bound water. This 15% DMA content has no improving effect upon catalysis compared to just 1% DMA. From all the conditions tested, both cutinase and Texazym PES showed the highest activity with 1% DMA, in spite of the error margin.

The two enzymes showed different behaviours using 1 M glycerol and a combination of 1 M glycerol with 1% DMA (Fig. 2). In spite of the similar increase of K/S in the absence of additives and for 1% DMA, the units of esterase activity from cutinase were more than twice those from Texazym, in all assays. This could mean a saturation of cutinase under these media conditions. When glycerol was present in the reaction media, Texazym treatment did not lead to a better staining of acrylics, while cutinase performance was improved with respect to the treatment without additives. The presence of 1% DMA could not raise the staining level provided by glycerol.

Differences in K/S were observed when the samples were stained at 70°C. At 80°C, the method failed to detect the action of both enzymes on the surface of the acrylic, except for the treatment with cutinase without additives. The rise from 70° to 80°C, which is very near the glass transition temperature for commercial acrylic fibres, has a major impact in segmental mobility of polymer chains. As the dye molecules penetrate deeply into the fibre, the differences in K/S are diluted. Dye molecules can also react with other chemical groups present in the fibre, such as the initiator molecules used in acrylic polymerisation. The yield of this side reaction will be enhanced by temperature since more of these groups will be exposed causing the colour depth to be higher. The addition of dye molecules to hydroxyl groups that result from enzymatic catalysis is not improved in an equal extension because they are located on the surface of the fibre. Thus, the observed increase in K/S, at 80°C, for the cutinase treatment without additives is an exception and can not be easily explained. To confirm the dependency of relative increases in K/S with dyeing temperature, more studies are required.

3.3 X-ray diffraction

The X-ray diffraction patterns obtained for the different samples showed the characteristic reflection peaks of polyacrylonitrile homopolymer (JCPDS card no 48-2119) [32, 33]. The main peaks are characterised by a well-defined sharp and intense peak positioned at $2\theta \approx 16.8^\circ$,

characteristic of the (010) plane, and a second one, less intense at $2\theta \approx 29.4^\circ$, from the (300) plane. A third diffuse peak was also observed in all samples, located at $2\theta \approx 26.5^\circ$, attributed to the (210) plane. This peak was found to be a very broad and diffuse one, commonly associated with amorphous phases. To obtain an accurate fitting profile, it was always necessary to consider a fourth significantly diffuse peak, at an angular position $2\theta \approx 16.8^\circ$, which is, in fact, very similar to that of the previously mentioned peak. Although unusual, similar behaviour was also found by Valerio *et al.* [34]. This peak appearance might result from the introduction of the co-monomer vinyl acetate, which probably induces some defects in the lateral packaging of polymer chains and thus is responsible for this second “amorphous type” phase.

No significant differences (below 1%) in the values of crystallinity index were found between acrylic control samples and samples treated with the enzyme (Table 2). This was expected given the superficial nature of the enzymatic treatment of the fabric and, in the particular case of acrylic fibres, the preservation of an intact carbon skeleton of polymer chains.

The X-ray diffraction and scanning electronic microscopy studies were initially intended as negative controls on the modification of physical properties of acrylic fibres. In fact, there were no qualitative significant differences on the morphology between controls and enzyme-treated acrylic samples analysed by scanning electron microscopy (data not shown). The chemical modifications catalysed by cutinase/esterase possibly do not significantly affect the physical properties of acrylic fibres.

4 Concluding remarks

A great amount of work has been published in respect to cutinase stability [9, 15, 24–26, 35–40]. Special attention has been devoted to surfactants, given the industrial interest in this enzyme for detergents formulation, for enzyme immobilisation and micro encapsulation, and for trehalose stabilisation. The study reported here con-

Table 2. Crystallinity degrees for acrylic fabric samples treated for 3 h with $9 \text{ U}\cdot\text{mL}^{-1}$ cutinase, at pH 8 and 30°C

Sample		Crystallinity degree	
No discs	No additives	Control	86.5
		Enzyme	86.3
Discs	No additives	Control	85.5
		Enzyme	86.2
	DMA 1%	Control	85.1
		Enzyme	85.0
	DMA 1% + glycerol	Control	85.7
		Enzyme	86.4

tributes to expand the pool of information regarding stabilisation additives for cutinase, in aqueous media, which is still of great importance in biotechnological applications.

The catalytic efficiency of both enzymes studied on acrylic surface modification, although not considerably significant, was proved to be enhanced by low concentrations of DMA, a solvent of PAN fibres commonly used in its spinning process. In addition, a reduction in incubation time with cutinase from 90 [6] to just 3 h, when a higher mechanical agitation was introduced, represents a great advance in the application of these enzymes to acrylic surface modification, especially in wet spinning lines of production. It is expected that the impact of esterase activity on fibre surface modification will be greater when used in acrylic or other textile fibres with higher contents in hydrolysable ester monomers in their compositions. Hopefully, in a near future, the use of enzymes will be routinely extended in an industrial scale to processes for staining and for improvement of comfort properties of synthetic fibres.

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