

# MODIFICATION OF YELLOW CORN STARCH ACETYLATION BY ACETIC ANHYDRIDE

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#### Abstract

Acetylation are chemical modifications which alter the properties of starch. The degree of modification of acetylated starches is dependent on the catalyst concentration the objective of this study was to evaluate the effect of acetylation on the structural, morphological, physical-chemical, thermal and pasting properties of corn starch. Corn starches were acetylated at catalyst (30% of NaOH solution) infrared spectroscopy (FTIR), X-ray diffractograms, thermal, morphological (SEM) and pasting properties, swelling power and solubility of starches were evaluated. The degree of substitution (DS) of the acetylated starches increased with the rise in catalyst concentration.

Key words: Yellow corn starch, acetylation, acetic anhydride.

#### Introduction

Starch is a polymer with a very complex structure, formed by glycosidic linkages between glucose units and presenting functional properties which make the polymer very helpful for the paper industry, the textile industry and mainly the food industry (Biliaderis, 1991). The food industry is the greatest consumer of starch, where it is used mainly because of the viscosity, gelation power, adhesion and retrogradation trend, among other properties, which are influenced mainly by the amylose to amylopectin ratio (Cereda & Carvalho, 2002). Amylose is one of the components responsible for the grain structure and its quantification is very important to understand starch behavior. In the food industry, starch is utilized to produce various functionalities such as thickening, stabilizing, texturing, gelling, encapsulation and shelf-life extension.

It plays an important role in determining the quality and texture of many foods; controlling the acceptability and palatability of most food products. Despite their advantageous properties, some starches in their native form may pose problems. For example, the tendency of its viscosity to increase rapidly and be thickened during heat treatment may cause difficulties in industrial food

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unit operations such as in pumps and heat exchangers. Some unmodified starches also have drawbacks such as low shear-stress and thermal resistance, thermal decomposition and high degree of retrogradation which limit their use in industrial food application (Singh *et al.*, 2004). Such undesirable characteristics may be overcome by modifying the starch (Bemiller, 1997).

Modification does not alter the appearance of the starch but can improve the desired properties of the starch. The most common method of starch modification is acetylation, i.e. esterification usually with acetic anhydride. Acetylation replaces hydroxyl groups in the native starch with acetyl groups. According to Rutenberg and Solarek (Rutenberg and Solarek, 1984a). Introduction of these acetyl groups reduces the bond strength between starch molecules and thereby alters the properties. Starches can be modified in several ways to change their function as additives in the products. Most commercial starches employed in either food or industrial applications have been chemically modified to improve their functionality (Zhang and Jackson, 1992).

Acetylation of starch provides stability and functional properties such as hydrophobic, cationic or anionic character at relatively low cost (Rutenberg and Solarek, 1984b). Acetylation of starches is an important substitution method that has been applied to starches that impart the thickening needed in food application. Introduction of acetyl group has improved properties over its native form and has been used for its stability and resistance to retro gradation. Acetylated starches with low degree of substitution (DS) are widely used in food industries for many years because of their important characteristics such as low gelatinization temperature, high swelling and solubility and good cooking and storage stability, increased viscosity, hardness and cohesiveness (De Graaf et al., 1995; Wang and Wang, 2000; Raina et al., 2006). Starch acetate was reported to be considerably more hydrophobic than its native starch and has been shown to have better tensile property retention in an aqueous medium, improved solubility compared to native starch and is easily cast into films from common solvents. The degree of acetylation is easily controlled by transesterification (Parandoosh and Hudson, 1993).

Therefore, this study aims to investigate the effect of different catalyst concentrations in the acetylation reaction of acetic anhydrite on the properties of corn starch, since we did not find similar studies. Also, the effects of acetylation on the structural, morphological, physical-chemical, thermal and pasting properties of corn starch were evaluated.

#### **Materials and Methods**

#### Materials

Corn starch, sodium hydroxide (NaOH), potassium hydroxide (KOH), absolute- ethanol, acetic anhydride, Acetone, hydrochloric acid (HCL), acetic acid, Iodine(I2), potassium iodide(KI), potassium bromide(KBr), phenolphthalein(ph.ph), methyl -orange, these chemicals were imported from abroad by Gamma Group for Medical and Laboratory Services) 5 Sheikh Ali Youssef Street, Kasr El-Ainy, Cairo, Egypt). All the chemicals and reagents used in this work were of analytical grade.

#### Methods

#### • Acetylation of starch:

Starch acetylation was performed according to method described by (Xu *et al.*, 2010) with some modifications. One hundred gram of starch were weighed (dry basis), dispersed in 200 mL of acetic anhydride in a reaction flask and stirred at 500 rpm with a mechanical stirrer for 5 min. Sodium hydroxide solution was used as the catalyst. The 30% NaOH solutions (50 g NaOH/100 g water) were added slowly to the starch suspensions with acetic anhydride. The NaOH concentrations (30%) were calculated based on the amount of starch. Each reaction was conducted at 100°C and stirred for 1 h.

The flask was then taken out of the container till the reaction medium reached 50°C. Starch was further precipitated with 100 mL of ethyl alcohol solution (96%). It was immediately filtered by suction with a Buchner filter funnel (Whatman filter No. 4). The residue was washed with ethyl alcohol and then with distilled water till most of the acetic anhydride was removed. The resulting paste produced by these washes was dried at 40°C for 16 h up to approximately 9% moisture content.

# • Determination of degree substitution of acetylated starch:

The acetyl group (AG expressed as percentage on dry basis) and the degree of substitution (DS) of corn starch were determined according (Mark and Mehltretter, 1972). A 5g of sample of starch was weighed, transferred to a 250 ml conical flask and dispersed in 50 ml distilled water. Few drops of phenolphthalein indicator were added and titrated with sodium hydroxide 0.1N to permanent pink color. Then 25.0 ml of 0.45N NaOH was added to it and shaken vigorously for half an hour. The stopper and neck of flask was flushed with little distilled water and then the excess alkali was titrated.

With 0.2N HCl to disappearance of pink color. A total of 25.0 ml of 0.45N NaOH was titrated as blank. Acetyl group and degree of substitution were calculated as follows:

% Acetyl =  $\frac{(\text{blank - sample})ml \times M_{HCL} \times 0.43 \times 100}{\text{Weight of sample}}$ D.S. =  $\frac{162 \times \% Acetyl}{4300 - (42 \times \% Acetyl)}$ 

# • Fourier Transform Infrared (FT-IR) spectroscopy determination of native starch and modified starch:

The FT-IR spectrum of native starch and modified starch were acquired on a Perkin Elmer FT-IR spectrophotometer (Perkin Elmer, Inc., MA, USA) using a potassium bromide (KBr) disc prepared from powered samples mixed with dry KBr. The spectra were recorded (16 scans) in the transparent mode from 4000 to 400 cm<sup>-1</sup> (Bernardino-Nicanor *et al.*, 2017).

# • X-ray diffraction:

A PANaLytical X-ray Diffraction equipment model X Pert PRO with Monochromator, Cu-radiation ( $\lambda$ =1.542 A°) at 45 k.v., 35, M.A. and scanning speed 0.03°/sec. were used. The reflection peaks between 20 = 2° and 60°, corresponding spacing (d, A°) and relative intensities (1/I°) were obtained. The diffraction charts and relative intensities are obtained and compared with ICDD files., the analytical by center by method from description in

# (Zobel et al., 1988).

#### • Scanning Electron Microscopy (SEM):

The size, shape, surface, crystalline structure and morphological data of native and modified starch granules was examined by Scanning Electron Microscopy (SEM) (JEOL TEM-2100) fixed to a CCD camera at an accelerating voltage of 200 kV. Each sample was prepared by suspending the sample on copper-coated carbon grids and the solvent was allowed to evaporate slowly before recording the TEM images. TEM measurements were recorded at the Central Laboratory, Electronic Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt (Vanier *et al.*, 2012).

#### • Molecular Weight (M.W):

The molecular weight of EPS (3&5) was determined on an Agilent 1100 HPLC system equipped with a refractive index detector (RID) and FPl gel particle size  $(5\mu m)$ , 3 columns of pore type (100, 104 and 105A°) on series, length  $7.5 \times 300 \text{ mm}$  (1000, 5000000) for DMF solvent Styrogel HR-DMF, 3  $\mu$ m (7.8  $\times$  300 mm), Water Company Ireland. One column (5000-600000) for water solvent (polyethylene oxide/glycol standard) PL aquagel-OH 7.5 mm and 30µm pore type 8 um particle size. The sample 0.01 gm was dissolved in 2 ml of solvent and then it filtrated by siring filter 0.45 then the sample put in GPC device (Xie et al., 2009). Number average molecular weight (MW) and weight average molecular weight (MW) were directly calculated according to the definition of Mn and MW using molecular weight and RI signal values at each elution volume (Kim and White, 2013).

• Swelling power:

Swelling power were determined by using Leach method (Leach, 1959). The 0.1 g samples (native and modified starch) were heated in 10 ml distilled water in a water bath at 60°C for 30 minutes with constant mixing. The samples were centrifuged at 1600 rpm for 15 minutes. The precipitated part was weighted and calculated using equation:

Swelling power = 
$$\frac{Weight of sedimental paste (g)}{Weight of the sample (dry basis) (g)}$$

#### • Water solubility:

Water solubility were determined using Kainuma method (Kainamu *et al.*, 1967). The 0.5 g samples (native and modified starch) were heated in 10 ml distilled water bath at 60°C for 30 minutes without mixing. The samples were centrifuged at 1600 rpm for 10 minutes. The supernatant was separated (5 ml), dried, weighted and

calculated using equation according to Varatharajan *et al.*, (2010).

% Solubility = 
$$\frac{Weight of the soluble starch (g)}{Weight of the sample (dry basis) (g)} \times 2 \times 100$$

# • Determination of amylose content:

The amylose contents (%w/w) from (native and modified starch) were determined by the spectrophotometric standard method (Williams *et al.*, 1970). A total of 100 mg of starch granules was homogenized with 1 ml of 95% ethanol and 9 mL of 1 M NaOH. The sample was heated for 10 min in aboilingwater bath to gelatinize the starch. After cooling, it was transferred into a volumetric flask and the volume was made up to 100 mL with water. Then 1 mL of 1 M acetic acid and 2 mL of iodine solution (0.2% I2, 2% KI) were added to a 5 mL aliquot. The solution was made up to 100 mL with water and allowed to stand for 10 min. Spectrophotometric quantification was performed at 620 nm, with a Shimadzu model UVPC 1601 spectrophotometer

Absorbance of blank solution prepared accordingly was subtracted from that of the sample and amylose content was calculated using equation according to International Organization for Standardization, (1987).

Amylose content (%) =  $3.06 \times \text{absorbance} \times 20$ 

# • Determination of Amylopectin content (AP):

Amylopectin content was calculated using the following equation explained by International Organization for Standardization, (1987). The average amylose content value was taken for the calculation:

Amylopectin (%) = 100 - Amylose content %

#### • % Degree of Gelatinization (DG):

DG (%) was calculated by a method of (Wootton *et al.*, 1971). For this, 0.2g sample (native and modified starch) was dispersed in 100 mL distilled water with stirring for 5 min and centrifuged at 1500 rpm for 25 min. One milliliter supernatant was then diluted to 10 mL with distilled water and 0.1 mL iodine solution was added. The method was repeated using 100 mL of 10 M potassium hydroxide instead of water and absorbance of both solutions were read at 600 nm in a Spectrophotometer (Cecil Aquarius 7400, England).

DG (%) = 
$$\frac{\text{Absorbance of fresh solution}}{\text{Absorbance of alkali solubilized solution}} \times 10$$

#### • Colour measurement:

The colour of all samples was determined in a Colour Measurement Spectrophotometer (Ultrascan Vis, Hunter Color-Lab, Virginia) according to (Sareepuang *et al.*, 2008). The result was expressed as L, a, b using corresponding native rice samples as reference. The chroma value (C) of parboiled starch was calculated.

 $C = (a^2 + b^2)^{1/2}$ 

# • Sediment volume:

Sediment volume of (native and modified starch) was determined with slight modification to the method of (Das *et al.*, 2010). Starch (1g, dry basis) was weighed into beaker and 95 ml of distilled water was added. The pH of the starch slurry was then adjusted to 7.0 using 5% NaOH or 5% HCl following which the slurry was cooked in a boiling water bath for 15 min. The mixture was then stirred thoroughly and transferred to a 100 ml graduated cylinder, the volume was made up to 100ml with distilled water. The cylinder was then sealed and kept at room temperature for 24 hrs for settlement of starch granules. The volume of the sediment consisting of starch granules was then measured for sediment volume.

# • Paste clarity:

The clarity (% transmittance at 650 nm) of (native and modified starch) paste was determined with slight modification to the method described by (Sandhu, K.S., and Singh, 2007). A 1% aqueous suspension of starch adjusted to pH 7.0 was heated in boiling water bath for 30 min with intermittent shaking. After that the suspension was cooled down to 25°C. The light transmittance was read at 650 nm against water blank.

#### • Statistical analysis:

Analytical determinations for the samples were performed in triplicate and standard deviations were reported, except for X-ray peaks and relative crystallinity and thermal analysis. Means were compared by Tukey's test at 5% level of significance by analysis of variance (ANOVA) (El-Halal *et al.*, 2015).

# **Results and Discussion**

# Acetyl Content (%) and Degree of Substitution (DS)

The starch extract was obtained from the interaction



 Table 1: Acetyl percentage, degree of substitution of acetylation-treated starches.

Acetylation Starches	Acetyl (%)	Degree of substitution	
Native	0	0	
30%NaOH	$8.428 \pm 0.08c$	$0.346 \pm 0.00c$	

of corn starch with acetic anhydride, in the presence of a sodium hydroxide as a catalyst. The acetylation ratio was 8.42 and the substitution degree was 0.34 as shown in table 1. It was found that by increasing the concentration of the sodium hydroxide catalyst to 30%, the ratio of acetylation and also the degree of substitution increased, compared to the results of the experiment research. And when conducting investigative tests for acetylated starch, it showed an improvement in the properties of the acetylated starch from the native starch. Where the degree of solubility, clarity and the low temperature required for gelatinization and low viscosity with the high temperature increased, the adhesive strength increased and the viscosity increased by increasing the straight chains in amylose from short ones.

# Fourier Transform Infrared (FT-IR) spectroscopy determination of native starch and modified starch



# FTIR of acetylated starches

To detect the structure of acetylated starches, FTIR spectra are recorded and the spectra of the starch, DS 0.346 acetylated starches are shown in fig. 1. In the spectra of acetylated starches, there are several discernible absorbancies at 1159, 1082, 1014 cm<sup>-1</sup>, which were attributed to C–O bond stretching (Goheen and Wool, 1991). Additional characteristic absorption bands appeared at 992, 929, 861, 765, 575 cm<sup>-1</sup> due to the entire anhydroglucose ring stretching vibrations. An extremely broad band due to hydrogen bonded hydroxyl groups appeared at 3421 cm<sup>-1</sup> (Fang, Fowler, Sayers and Williams, 2004). FTIR spectra of high DS of acetylated

starches showed some new absorption bands at 1754, 1435, 1375, 1240 cm<sup>-1</sup> assigned to carbonyl C=O, CH3 antisymmetry deformation vibration and CH3 symmetry deformation vibration, and carbonyl C–O stretch vibration, respectively. These new absorptions suggest that the acetylated starch products were formed during the esterification process. In addition, the spectra of acetylated starch showed that



the anhydroglucose unit moved towards a high wave number. With DS increasing, the intensities of peaks at 3421, 1082, 1014 cm<sup>-1</sup> were gradually weakened and almost disappeared when DS reached to 0.346, indicating hydroxyl groups were just about to participate in the reaction.

# X-ray diffraction

X-ray diffraction measurements were performed to check if chemical modification altered the crystallinity of starch. The X-ray diffraction spectra of native starch and acetylated starches are presented in fig. 2. Sharp diffraction peaks at 15, 17, 18, 23 (2h), which indicated typical acetylated starches pattern of cereal starch (Zobel, 1988). As can be seen in fig. 2, DS 0.346 acetylated starch showed a similar profile of native one, but it had a new peak at 9 (2h), which appeared diffusion peaks of acetylated starch. However, DS 0.346 acetylated starches represented typical peaks of acetylated starches, which had wide peaks at 9 and 20 (2h). The X-ray diffraction indicated that with esterification processing, crystalline structures of native starch were destroyed and new structure of acetylated starch was formed. (Zobel, 1988).

# Scanning Electron Microscopy (SEM)

SEM micrographs of acetylated starch are shown in fig. 1 at 100 nm. Most acetylated starch granules were oval and had a smooth surface. Some granules were globular or irregular in shape and there were cavities on the surface of some starch granules. Our observations were consistent with previous reports (Shao *et al.*, 2010; Zhang *et al.*, 2019; Li *et al.*, 2020).

As shown in fig. 1, the size of acetylated starch granules could vary greatly ranged from 71.57 to 129.38 nm. Previous studies suggested that the size differentiation of starch granules could bring significant variation in starch physicochemical properties (Li *et al.*, 2020).



Based on our preliminary data (Fig. 1), large

Table 2:	(FT-IR)	spectroscopy.
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No.	Position	Intensity
1	3947.57	81.1776
2	3455.81	40.5894
3	2928.38	69.352
4	1732.73	71.6741
5	1635.34	70.2395
6	1378.85	69.2031
7	1257.36	67.4775
8	1037.52	60.9624
9	700.034	72.1014
10	582.397	72.4684

intermediate and small grains of acetylated starch showed significant difference in gelatinization temperature. A further detailed study will be conducted to elucidate the difference in starch physicochemical properties in relation to starch granule size differentiation.

#### Molecular Weight (M.W.)

In contrast to amylose content, the values of weightaverage molecular weight of the studied starches have shown significant differences. We found that the molecular weight of rice starch is the highest reaching a value of 83.2 MDa. Potato starch presents an intermediate value (69.5 MDa) and corn and wheat starches have similar and the lowest values (≈51 MDa). The origin of these values could be in the molecular weight of the two macromolecular constituents of starch, amylose and amylopectin. As stated, amylose is a linear polysaccharide with an average molecular mass of 105 g·mol<sup>-1</sup> and amylopectin is a branched polysaccharide with an average molecular mass of 106-107 g·mol<sup>-1</sup>. For this reason, starches with high amylose content could presumably have lower molecular weight and a relatively more linear structure than those with a high content of amylopectin. Luchese et al., (2018).

#### Swelling Power and Solubility

Swelling Power and Solubility Swelling power and solubility at 80°C of acetylated corn starch with degree of substitution (0.34) were in the range of 44.32 g/g and 27.66%, respectively see table 4. These values are higher than for the native starch (9.03 g/g and 2.73%, respectively). This increase in swelling power and solubility for an acetylated starch is consistent with observations made by other researchers J. Singh, L. Kaur and N. Singh.

#### **Amylose and Amylopectin content**

Amylose and Amylopectin Content

Table 3:	X-Ray.
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Peak	Pos.	d-spacing	Height	FWHM	Rel.Int.
No.	[°2Th]	[?]	[cts]	Left [°2Th]	[%]
1	15.3439	5.77477	30.91	0.7872	77.24
2	23.1440	3.84316	40.02	0.6298	100.00

Starch consists of two fractions, namely amylose and amylopectin. Both macromolecules greatly contribute to the physical, chemical and functional properties of the starch. Amylopectin has a branched chain of properties and is easily degraded by digestive enzymes, whereas amylose is not easily degraded by enzymes. Based on our observations (Table 4), the amylose and amylopectin contents of the modified starch had various values. The amylose 24.76%, while the levels of amylopectin 75.24%. The high amylose levels of corn starch are considered to provide good opportunities for modification of the starch. Native corn starch has elevated levels of amylose (24.76%) and amylopectin (75.24%), gave the process of a acetylation corn starch effect to changes in the composition of amylose and amylopectin in the starch granules (Table 4). The tendency observed was that amylose content measured higher than its native form when a concentration of 26.84% cataliz reported by Singh, et al. the presence of certain chemical groups (such as the acetyl group or other groups) can lead to increased estimated levels of amylose



Fig. 3: SEM micrographs characterization illustrating the size and morphology of starch at 100 nm.



Native Acetylated Starch Starch Swelling power 9.03 44.32 2.73 27.66 Water solubility Amylose content 24.76 26.84 Amylopectin content 75.24 73.16 177.083 % Degree of gelatinization 68.3 L=80.72 L = 83.28Colour measurement A=1.17, B=6.15 a = 0.45b = 8.60 $C^* = 7.19$ Sediment volume 58.9 32.8 Paste clarity 30.15 45.81

# % Degree of gelatinization

The % degree of gelatinization in the temperature range of 50-95°C was followed by the spectrophotometric method based on the formation of amylose-iodine complex. In fact, % DG based on measurement of absorption peak at 600 nm can be taken as the ratio of solubilized amylose sites to the total amylose sites available in acetylation starch. Taking this point into account. The % degree of solubilized amylose sites to the total in the native starch was higher for acetylated starch, at lower temperatures, it increased after 80°C. If the amylose was the only soluble portion in starch, similar behaviors should

be observed in %DG at 95°C indicated that mainly the amylopectin forms the soluble portion, since it only contains 1-2% amylose. Moreover no blue color but red coloration with iodine must be considered as an indication of amylopectin in soluble portion. Using %DG data at 95°C and taking amylose contents of starches into account, the percentage of amylose in the soluble portion has been calculated. The results obtained showed that the soluble portion of contained 24.76% amylose by weight. acetylated starch was %DG (177%) and the soluble portion of acetylated starch nearly contained only amylose. This was also evident from the gels obtained. When cooked and formed soft gels. In contrast, high amylose gels (normal corn starch and acetylated starch) were firm because amylose contributes gel strength and firmness and results in a tighter network. E. Aytunga Arik Kibar, Ilknur Gönenç, Ferhunde Us (2009).

**Colour measurement** 

As shown in table 4, Acceptable degree of whiteness was observed for corn starch with L\* value of 83.28. Boundries et al., (2009) have concluded that "L" values greater than 90 give a satisfactory whiteness for starch purity. The degree of redness represented by the positive value of "a" 1.17 the starch and Also, the value "b" indicates yellow the positive "b" value of 6.15 noticeably small he pointed out that the yellowing showed a moderate decrease in the treated samples, but they remained Almost unchanged with treatment intensity. Changes in color values were not significant in a way that affects color Decrease in the value of "C" was due to a decrease in the value of b when soaking to separate the components of the pill. The change in redness and yellowing is noticeably correlated, which may affect the final value of c, as well as the degree of whiteness.

#### Sediment volume

The sediment volume of corn starch is 76.88 ml as presented in table 4. corn starch is having highest sediment volume and upon modification of acetylation starch. There is high in sediment volume. The high values in modified starch are due to the disruption of granules resulting in high swelling and volume makeup. Studies were done for acetylation corn starch which reported high sedimentation volume, due to high interaction between starch molecules by and High swelling by acetylation. The high sediment volume may also be due to large starch granules which caused high in bond strength upon heating. Raina *et al.*, (2006).

#### Paste clarity

Acetyl groups disrupt interactions among outer chains of amylopectin, the branched form of starch and among amylose chains Decreased interactions prevent cloudiness and syneresis in aqueous starch dispersions stored at low temperatures. This effect is evident in the present study also showing that after modification, the paste clarity of starch increased as compared to the native starches. The paste clarity increased with increase in DS level. However, in, having relatively higher amylose content showed the lower extent of increase in paste clarity upon modification as compared with native starch. Jeong *et al.* 

# Conclusions

Previously we could conclude that acetylated yellow corn starch and its morphology, size distribution and physicochemical properties have unique characteristics were observed. Conducting investigative tests for acetylated starch showed an improvement in the properties of the acetylated starch from the native starch. Where the degree of solubility, clarity and the low temperature required for gelatinization and low viscosity with the high temperature increased, the adhesive strength increased and the viscosity increased by increasing the straight chains in amylose from short chains. The starch granule size varied widely, even in the same sample. Further studies should be conducted to compare the starch physicochemical properties among native and acetylated starches of different sizes, large, intermediate and small. In summary, the results reported by the present study should inspire further studies to explore the potential of acetylated starches for utilization in food and non-food industries.

# Application

Bulking agent in snack foods, stabilizer and thickener in most foods, improves smoothness and sheen of soups and sauces. Cholesterol-free salad dressings and flavor encapsulating agents in clouding agents, creamer and beverage. Substitute to gum arabic, egg yolk and caseinates Korma *et al.* and or the paper industry, the textile industry.

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