A Fast and Selective HPLC Determination of Ulose Formation during Enzymatic Isomerisation of Pentose Rich Syrups

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Abstract :

Xylose, and arabinose rich syrups obtained by hydrolysis of wheat bran represent a large potential as fermentation substrates in the production of *second generation biofuels* (1), providing that pentoses are isomerised into the 2-ulose form previously, or during in the fermentation process.

The transformation of arabinose and xylose present in pentose rich syrups into the corresponding ketoses using immobilised isomerases produces a very effective fermentation medium. (2).

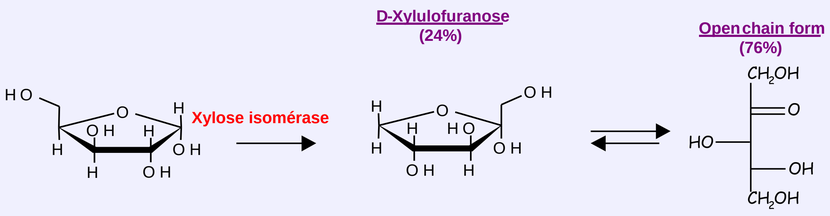
Existing analytical protocols to monitor the isomerisation rate, such as the carbazol test (3) or the resorcinol method (4) do poorly on concentrated syrups containing a mixture of various types of aldoses, especially at low and medium ulose concentrations.

In order to closely monitor the action of the isomerase on the sugar mixture we developed a new fast and selective high performance liquid chromatographic (HPLC) analysis of the produced uloses, directly in the concentrated complex carbohydrate mixtures (5).

This method uses the UV absorption specificity of the open-chain ketose form at 210 nm. The test is linear up to a concentration of 20 g/L ketose even in the presence of 50 g/L D-xylose or L-arabinose.

The sensitivity of th method is 0.5 g/L for D-xylulose and L-ribulose.

1) Pomeranz, Y., 1988. Chemical composition of kernel structures. In: Pomeranz, Y. (Ed.), Wheat Chemistry and Technology. AACC, St Paul, MN, pp. 97–158.
2) Chandrakant, P.; Bisaria, V. S. Appl. Microbiol. Biotechnol.2000, 53, 301–309. & Dien, B. S.; Cotta, M. A.; Jeffries, T. W. Appl. Microbiol.Biotechnol. 2003, 63, 258–266
3) Dische, Z.; Borenfreund, E. J. Biol. Chem. 1951, 192, 583–587.
4) Kulka, R. G. Biochem. J. 1956, 63, 542–548.
5) Givry, S.; Bliard, C.; Duchiron, F. Carbohydrate Research 342 (2007) 859–864



Introduction:

Industrial wheat bran is composed of various layers of the grain i.e. the external coverings, the aleurone layer and the remainders of the starchy endosperm and usually accounts for approximately 20 % of the grain.

Wheat bran Can be chemically or enzymatically hydrolyzed into a mixture of a variety of hexoses and pentoses, including glucose, mannose, galactose, xylose, and arabinose (1).

However the majority components of wheat bran syrup are the xylose, glucose and the arabinose respectively by order of importance.

The increasing interests in second generation biofuels in the last ten years have seen the emergence of numerous examples allowing the use of pentoses for the production of ethanol. In these processes of arabinose and xylose isomerization into the corresponding ketoses with isomerases it is necessary to carry out the fermentation simultaneously to prevent back-up mechanisms (3) or include the isomerase activity in the genetic construction as described in the *Zymomonas mobilis* or *Saccharomyces cerevisiae* strains (4)

Different protocols such as the carbazole test, originally described by Dische and Borenfreunds (5), or theresorcinol method of Kulka (6, 7) can be used for the detection of these isomers but only in single carbohydrate containing media and its corresponding isomer. Various HPLC methods have been described for the determination of these carbohydrates but none theses methods allow a satifactory separation of all individual sugars of a complex medium.

Other methods such as gas chromatography and HPAEC or IR spectroscopy for the determination of fructose and glucose have been developed for the detection of these carbohydrates but for complex biological mixture these methods are not adapted. Proper samples preparation is undoubtedly the primary difficulty encountered.

Furthermore, none of these methods allow the determination of small quantities of ketose isomers such as ribulose or xylulose in the presence of large quantities of the corresponding aldoses.

1) Sues, A.; Millati, R.; Edebo, L.; Taherzadeh, M. J. *FEMS Yeast Research* 2004
2) Chandrakant, P.; Bisaria, V. S. *Appl. Microbiol. Biotechnol.* 2000, 53, 301-309.
3) Dien, B. S.; Cotta, M. A.; Jeffries, T. W. *Appl. Microbiol. Biotechnol.* 2003, 63, 258-266.
4) Deanda, K.; Zhang, M.; Eddy, C.; Picataggio, S. *Appl. Microbiol. Biotechnol.* 1996, 62, 4465-4470.
5) Dische, Z.; Borenfreund, E. *J. Biol. Chem.* **1951**, 192, 583 - 587.
6) Kulka, R. G. *Biochem. J.* **1956**, 63, 542-548.
7) Schenk, M.; Bisswanger, H. *Enzyme and Microbial Technology* **1998**, 22.

Aim and Scope :

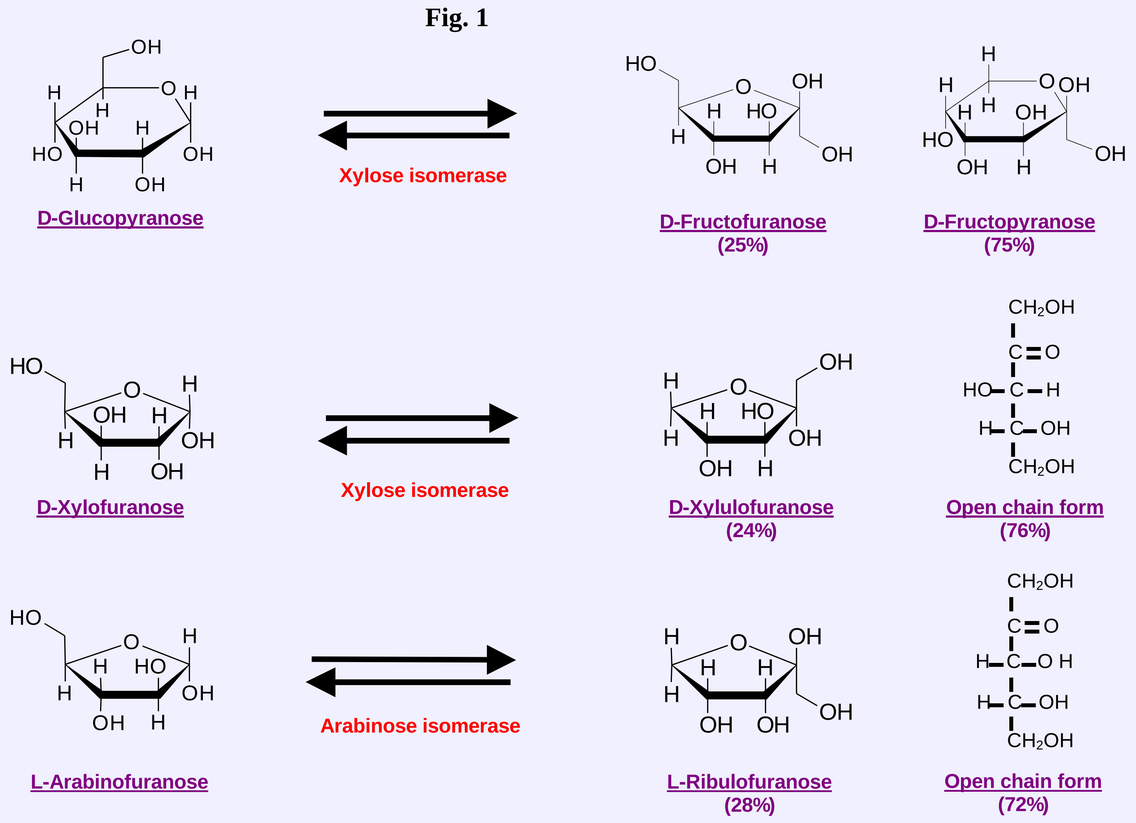
In the present study study we report the results of a HPLC protocol allowing the selective analysis of individual ketoses in complex mixture.

In pentoses two major isomeric structures are found:
- the hydroxyaldehydic one (aldoses) and the
- hydroxyketonic one (ketoses).

In pentuloses the six atom pyranosidic ring closing form is rendered impossible. Köpper and Freimund (1) reported that the major isomeric form under which both ribulose and xylulose were found in aqueous solution was the ketone containing open-chain form, the remainder being the mixture of the two anomeric cyclic furanoses [Fig. 1].
The presence of this free ketone function allowed us to carry out the selective UV detection of ribulose and xylulose.

1) Köpper, S.; Freimund, S. *Helvetica Chimica Acta* **2003**, 86, 827-843.

Fig. 1



Material an Method

The chromatographic analysis was carried out in a Beckman HPLC chromatograph using a Programmable Solvent Module 126 Pump, and an autosampler 502 equipped with a 20 µl loop, (Analogue Interface Module 406 and the Gold 4.0 controller software).

The chromatographic separation was performed on 300 x 10 mm chromatographic column. Aminex HPX-87H, HPX-42A (BioRad Lab., Richmond, Calif. USA) and Metacarb 87P (Varian, LesUlys, France).

MilliQ water with a specific resistance higher than 18.2 Ω was used in the mobile phase and the for the concentrated sample preparation. The samples were filtered through a 0.22 µm porosity cellulose acetate prior to injection.
The flow rate was set to 0.6 mL/min and the separation temperature was regulated at 65 °C. Supelcosil® (Supelco, Bellefonte, PA) with acetonitrile (75%) as a mobile phase at a flow rate of 0.6 mL/min and the separation temperature being regulated at 85 °C.

Two detectors: a variable wavelength ultraviolet Beckmann Programmable Solvent Detector 166 and a Iota 2 refractive index detector (Precision Instruments, Marseille, France) were used for the simultaneous detection of the hexo- and keto-carbohydrate compounds. Peaks were measured at 190 to 220 nm, respectively.

L-arabinose, D-glucose, D-fructose, D-xylose, D-xylulose, D-ribulose, were used as standards. Highly concentrated samples were prepared by diluting with MilliQ watersolution over a Microcon Centrifugal 10 kDa Filter Devices.

Results
The results obtained are shown in the Table 1.

Table n° 1: Retention time of standards on various columns with the respective eluent.

Column	Eluent	Carbohydrate					
		Arabinose	Glucose	Xylose	Ribulose	Fructose	Xylulose
Aminex-87H	5mM H_2SO_4	11'33	9'625	10'473	11'428	10'226	10'879
Aminex-87P	H_2O	17'574	13'886	15'047	ND(*)	19'16	18'102
Aminex-42A	H_2O	18'145	16'44	16'451	17'93	17'403	16'839
Supelcosil	75% CH_3N	15'908	18'29	14'741	13'359	16'61	13'453

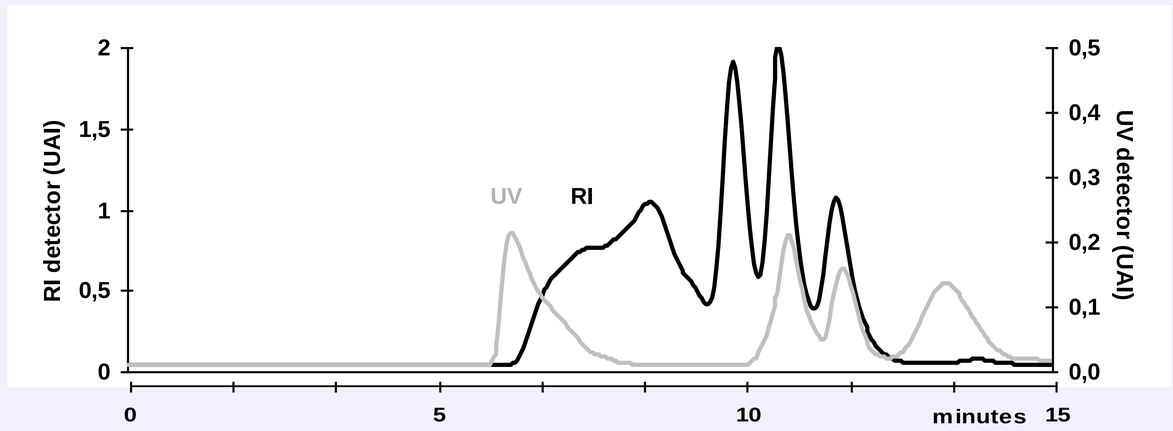


Figure n° 2: Comparative RI and UV detection at 210 nm during separation of arabinose, xylose and glucose from wheat bran syrup with a final concentration of 100 g/L and addition of ribulose and xylulose to a concentration of 2 g/L on an Aminex HPX 87H column at 45°C with water at a flow rate of 0.6 mL/min. (RI x 32)

Conclusion

The method described presents an economical, rapid, and accurate technique for the detection of high concentration of carbohydrate such as glucose and pentoses, and corresponding the ketoses at lower concentration.
The process provides a mean to quantifies simultaneously various ketoses such as ribulose and xylulose in the processes of isomerisation and simultaneous fermentation. The production of ketoses by isomerases and also their subsequent consumption by micro-organisms in a complex mixture of various sugars can be monitored.

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