STRATEGIES FOR THE ENZYMATIC DETERMINATION OF ETHANOL IN BEVERAGES USING FLOW BASED APPROACHES

Susana S. M. P. Vidigal, Teresa F. M. Pais, Ricardo N. M. J. Páscoa, Ildikó V. Tóth and António O. S. S. Rangel

Escola Superior de Biotecnologia, Universidade Católica Portuguesa
Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
e-mail: aorangel@esb.ucp.pt

DETERMINATION OF ETHANOL

Determination of alcoholic degree

Precision of reference methods:

on wine distillate : 0.04 -0.6% (v/v)

on nondistilled wine: 0.2% (v/v)

Fermentation monitoring

Precision of methods:

on nondistilled wine: ≈ 2 % (v/v)

ENZYMATIC DETERMINATION OF ETHANOL

Alcohol dehydrogenase (ADH)

$$CH_3CH_2OH + NAD^+ \xleftrightarrow{ADH} CH_3CHO + NADH + H^+$$

☑ Higher stability
☑ Better selectivity
☒ Need of cofactor

Detection method
- spectrophotometric
- fluorimetric
- amperometric

Alcohol oxidase (AOD)

$$CH_3CH_2OH + O_2 \xleftrightarrow{AOD} CH_3CHO + H_2O_2$$

☒ Lower stability
☑ Cofactor bounded to the enzyme

Detection method
- chemiluminometric
- amperometric
- spectrophotometric

Flow strategies

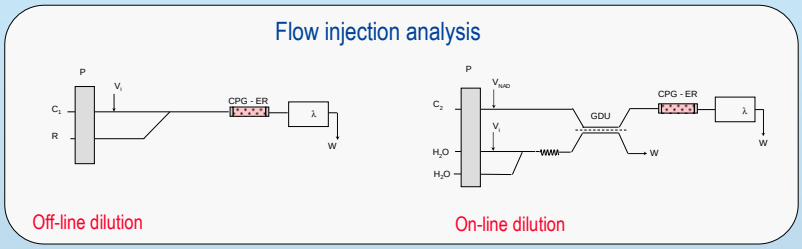

Figure 1. Flow injection manifolds for the enzymatic determination of ethanol in beverages, P, peristaltic pump; C_i, carrier; C_1 and C_2, carbonate buffer, pH 9; R, NAD^+ solution; V, valve; V_i , sample injection ; V_{NAD}, NAD^+ injection; GDU, gas diffusion unit; CPG-ER, immobilized alcohol dehydrogenase enzyme reactor; λ, spectrophotometer, 340 nm; W, waste.
António O. S. S. Rangel & Ildikó V. Tóth, Am. J. Enol. Vitic. 50 (1999) 259.

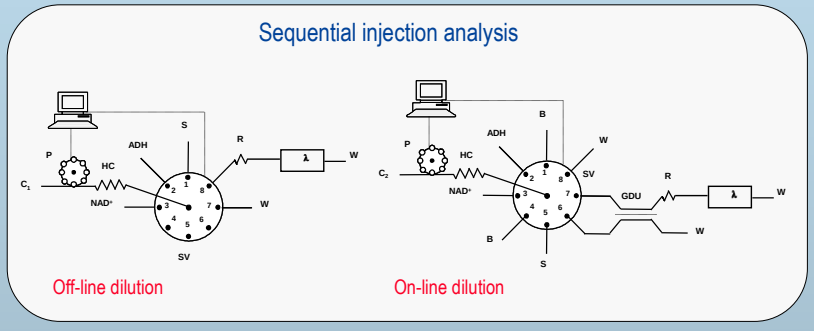

Figure 2. Sequential injection manifolds for the enzymatic determination of ethanol in beverages, P, peristaltic pump; C, carrier; C_1 buffer, pH 10; C_2, H_2O; S, sample; R, reactor; SV, selection valve; HC, holding coil; GDU, gas diffusion unit; ADH, alcohol dehydrogenase enzyme solution; B, buffer; λ, spectrophotometer, 340 nm; W, waste.
Ricardo Páscoa et al., J. Agric. Food Chem. 54 (2006)19-23.

Analytical figures of enzymatic (ADH) flow methods for ethanol determination in beverages

	Worsfold et al. 1981	Lazaro et al. 1987[a]	Rangel, Tóth, 1999	Rangel, Tóth, 2000[b]	Mori et al. 2003 [a]	Heddenfalk, Mattiasson, 1996	Segundo, Rangel, 2002 [b]	Chen, Ruzicka, 2004	Páscoa et al. 2006	Pais et al. 2006
Flow technique	FIA	FIA	FIA	FIA	FS	SIA	SIA	Lab on Valve	SIA	SIA
Sample matrix	beverages	wine, beer	wine	wine	wine, sake	ferment. broth	wine	no application	wine	wine
Sample dilution	off line	off line	on line	on/off line	off line	on line	on/off line	NA	off line	on line
Enzyme state	solution	immobilized	immobilized	immobilized	immobilized	immobilized	immobilized	solution	solution	solution
Reagent consumptions ADH (U/assay)	225	not given	≈0.4	not given	16.7	not given	not given	48	1.1	0.45
NAD^+ (μmol/assay)	2.4	3.2	0.6	14	0.52	0.5	0.3	0.7	1	1.3
Sample (μL/assay)	30	30	25	3000	50	150	45	30	100	32
Waste production (mL/assay)	1	1.8	11	8.1	5.2	not given	1.8	1	1.8	3.4
Determination rate (h^{-1})	120	40	30	20	10	26	45	120	25	21
RSD (%)	not given	±0.3	<2.2	<2.3	2	<6	<3.4	<3	<2.3	<3.4

a) with simultaneous determination of acetaldehyde; b) with simultaneous determination of glycerol

Conclusions

❶ Enzyme immobilisation does not necessary mean lower enzyme consumption.

❷ Achieved precision is more adequate for a screening method or for fermentation monitoring.

❸ Down scaling of the flow systems seems to affect precision.

References

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