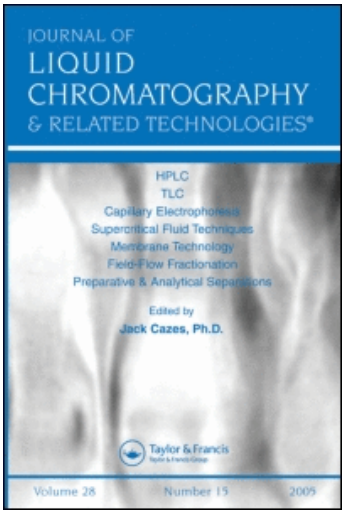

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DETERMINATION OF FURFURAL COMPOUNDS IN ENTERAL FORMULA

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ABSTRACT

HPLC methods are described for the determination of furanic compounds (hydroxymethylfurfural and furfural) in enteral formulas prepared with dextrinomaltose and milk proteins, and in model systems enclosing these ingredients. These compounds were extracted in aqueous solution, purified with organic solvents, and separated in a reversed-phase C_{18} column with water-acetonitrile (95:5 v/v). Average recovery rates of HMF and furfural were 99.2% and 71.1%, respectively. The variation coefficients for HMF and furfural were 2.41% and 1.23%, respectively. The detection limit was 0.01 mg/L for both compounds. HMF and furfural levels in enteral formulas ranged from 0.05 to 19.1 mg/L and from 0.14 to 0.72 mg/L, respectively.

INTRODUCTION

Enteral formulas are products with physico-chemical and biological properties that allow them to be administered through a tube into the gastrointestinal

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tract. Delivery of nutritional support with enteral feeding is expanding and sales of commercial preparations are rising. This development can largely be attributed to the introduction of simple and low risk procedures for placing the tube in the gastrointestinal tract, and to the wide variety of commercial enteral feeding formulas now available, offering a range of nutrient components.(1)

Complete formulas normally contain a specific combination of protein, fat, carbohydrate, vitamin, and mineral components. The proteins used are preferentially caseins and whey proteins, although, some formulas enclose soy proteins. The carbohydrates are mostly dextrinomaltose, glucose, maltose, or lactose. Manufacturing steps include the blending, pasteurization, homogenization, and sterilization of the materials. The application of heat treatment facilitates the preparation of the products, guarantees their safety, and prolongs their storage life.

One of the modifications induced by heating is the Maillard reaction, which involves amino acids and reducing carbohydrates, and can produce losses in nutritional value.(2,3)

Hydroxymethylfurfural (HMF) is an intermediate product in the Maillard reaction,(4) and is also formed from the degradation of hexoses heated in acid solutions.(4,5) HMF is a classic index of the browning process in milk, for which two main types are used: free HMF (formed by Maillard reaction and sugar degradation), and total HMF (coming from artificial degradation of lactulosyllysine through 1,2 enolization in the Maillard reaction).(6,7) In juices(8) and honey,(9) the main pathway is sugar degradation, because of the high concentration of sugar and low pH. HMF was determined in dried pasta by Acquistucci and Bassotti(10) and Resmini et al.,(11) and in breakfast and baby cereals by the present research group.(12-14)

Furfural is another product that derives from the browning reaction or L-ascorbic degradation.(15) Furfural has been widely used as a marker of the browning reaction in juices,(16-17) spirits(18) and infant milk formulas.(19)

According to our search of the literature, no data are available on furfural compounds in enteral formulas.

The aim of the present study was to develop a method for the determination of HMF and furfural in enteral formulas that could be used to control their processing.

EXPERIMENTAL

Apparatus

The liquid chromatographic system used in this study consisted of a Konic model 500A chromatograph (Barcelona, Spain) with 20 µL injection loop, Spherisorb S5 ODS2 (250 mm × 40 mm i.d.) column (Sugelabor, Madrid, Spain), Konic model 200 UV/VIS detector (Reno, NE), and Hewlett Packard model 3394A integrator (Avondale, PA).

For the diode-array study, the liquid chromatography system consisted of a Perkin-Elmer model 250 liquid chromatograph (Norwalk, CT), Perkin-Elmer model 235 diode array detector (Norwalk, CT), and LCI 100 Perkin-Elmer integrator (Norwalk CT).

The absorbance measurement was carried out with a Perkin Elmer model 551 S UV/VIS spectrophotometer (Norwalk, CT).

Reagents

All reagents used were of analytical grade. The clarified solution was composed of 15% potassium ferrocyanide (w/v) (Merck, Darmstadt, Germany) (Carrez I) and 30% zinc acetate (w/v) (Merck, Darmstadt, Germany) (Carrez II). A standard stock solution containing 200 mg/L 5-(hydroxymethyl)furfural and 2-furaldehyde (Merck, Darmstadt, Germany) was used to prepare the working standard solutions (0.01 - 0.5 mg/L).

Samples

Commercial Enteral Formulas

Eighteen commercial enteral formulas were assayed.

Model Systems

A mixture of casein-dextrinomaltose (5.46 g calcium caseinate, 11.20 g dextrinomaltose) and casein-lactose (5.46 g calcium caseinate, 5.71 g lactose) was brought to 100 mL with deionized water and magnetic agitation. Two aliquots of 10 mL were then placed in Pyrex-screw cup vials and heated at 120°C for 9, 15, and 30 minutes in a Selecta Bloc 12 mineralizer. Samples were then cooled in an ice bath and stored at –50°C until analysis. Similar model systems, heated at 120°C for 9 minutes, were stored at room temperature or 55°C for one month. The products were provided by a Spanish dietetic company.

HMF and Furfural Determination

Extraction

One mL of the sample was added to 4 mL of deionized water and clarified with 0.25 mL each of Carrez I and II solutions. The resulting mixture was cen-

trifuged for 10 min at 5000 rpm in a 10 mL centrifuge tube. The same procedure was followed twice more, adding 3 mL of deionized water to the pellet and shaking vigorously for 2 minutes. The supernatants were combined and the solution was brought to 10 mL (aqueous solution). The HMF and furfural studies were both used in this extraction procedure.

Purification and Concentration

a) HMF: 10 mL of the aqueous extract described above were added to 10 mL of trichloromethane and shaken vigorously for 2 min. The organic fraction was separated and the same procedure was followed 9 more times. Three mL of deionized water were added to the 100 mL of trichloromethane extract and evaporated under vacuum. The water fraction was filtered through an 0.2 μm disk filter before injection.

b) Furfural: 10 mL of the aqueous extract were added to 10 mL of diethyl ether and shaken vigorously for 2 minutes. The organic fraction was separated and the same procedure was followed twice more. Three milliliters of deionized water were added to the 30 mL of diethyl ether extract and evaporated under vacuum. The water fraction was filtered through an 0.2 μm disk filter before injection.

Chromatographic Conditions

Twenty microliters of filtered solution were separated in a reversed-phase C_{18} column. The mobile phase was water-acetonitrile (95:5) (Panreac, Barcelona, Spain) and the flow rate was 1 mL/min. HMF was detected at 284 nm in 8 min. Furfural was detected at 277 nm in 11 minutes. The run time was always 15 min.

The external standard method was used for the calibration. The HMF concentration range was 0.01 - 0.5 mg/L. The linear regression equation used was (n = 7) Y = 745112.386X - 1015.853, where Y is the peak area and X is the HMF concentration. The correlation coefficient was r^2=0.9999. The furfural concentration range was 0.01-0.5 mg/L. The linear regression equation used was (n = 7) Y = 998.9514X-2.656, where Y is the peak height and X is the furfural concentration. The correlation coefficient was r^2=0.9996.

Triplicate and quadruplicate samples were analyzed.

Statistical Analysis

Analysis with the Student's t test and linear regression equation was performed with a Sigma package (Horus Hardware S.A., Madrid, Spain).

RESULTS AND DISCUSSION

HMF and furfural are the most widely used furanic compounds for the assessment of non-enzymatic browning in foods.(19) However, to our knowledge, no data are available on the levels of furanic compounds in enteral formulas.

Clarifying Reagents

Trichloroacetic (40% w/v) and Carrez reagents, commonly used in milk and fruit juices, respectively, were assayed as clarifying reagents using acetonitrile-water as mobile-phase. Carrez was selected for the study, because of the absence of interference between peaks.

Chromatographic Conditions

Acetic-acetate buffer 0.08 M (pH 3.6), methanol-water, and acetonitrile-water, previously described for the determination of furfural compounds, (7,13,16) were assayed at different ratios as mobile-phases. The resolution obtained with all the phases was similar, although, a slight increase in 284 nm absorbance was obtained when the buffer phase was used. The spectrophotometric determination (at 284 nm) of an HMF solution prepared in water, acetonitrile-water, and acetic-acetate buffer showed absorbance ratios of 1.0, 0.9, and 1.07, respectively. Acetonitrile-water, was finally selected as the mobile phase for our study, because it provided adequate resolution and did not suffer the obstruction problems that arose when the buffer phase was used.

Purification Method

The chromatograms obtained for HMF and furfural after clarifying with Carrez appeared to showed a good resolution. However, the purity shown by the HMF and furfural diode-array spectra was inadequate (fig. 1).

The HMF purification study used two different organic solvents, trichloromethane, and benzene. Purification was always better with trichloromethane (fig. 2). Ten extractions were necessary to obtain 100% of HMF: after three extractions, 49% of HMF was obtained; after five, seven, and nine extractions, 70%, 85%, and 95% of HMF was obtained, respectively.

The furfural purification was assayed with trichloromethane and diethyl ether. Both solvents recovered 100% of furfural after three extractions. The evaporation of the organic solvents produced a loss of furfural that was smaller

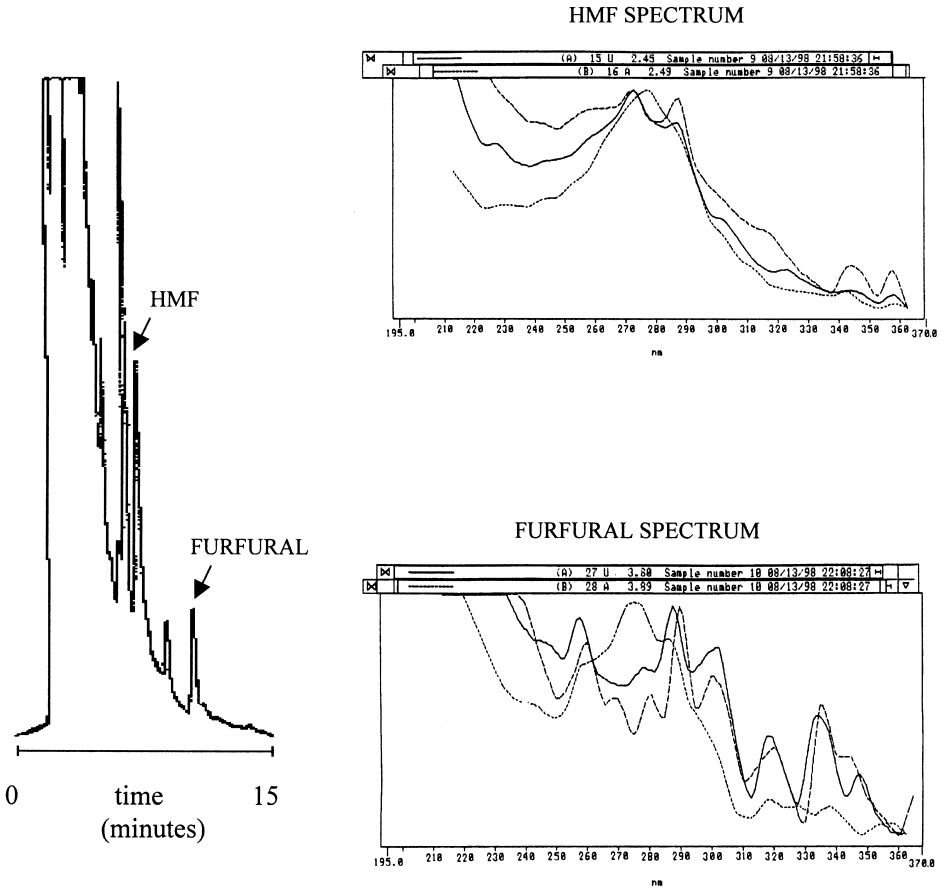

Figure 1. Spectra and chromatogram of unpurified furanic compounds of "R" enteral formula.

with diethyl ether (29%), yielding a total recovery of 71%. Figure 2 shows the chromatogram and diode-array spectra of furfural purified with diethyl ether.

These procedures allowed both the purification and the concentration of the samples.

Recovery

Recovery was determined by the standard addition procedure. Standards of HMF and furfural were added to one and two mL of "Q" sample and the recovery was approximately 20% higher for 1 mL than for 2 mL of formula. The HMF recov-

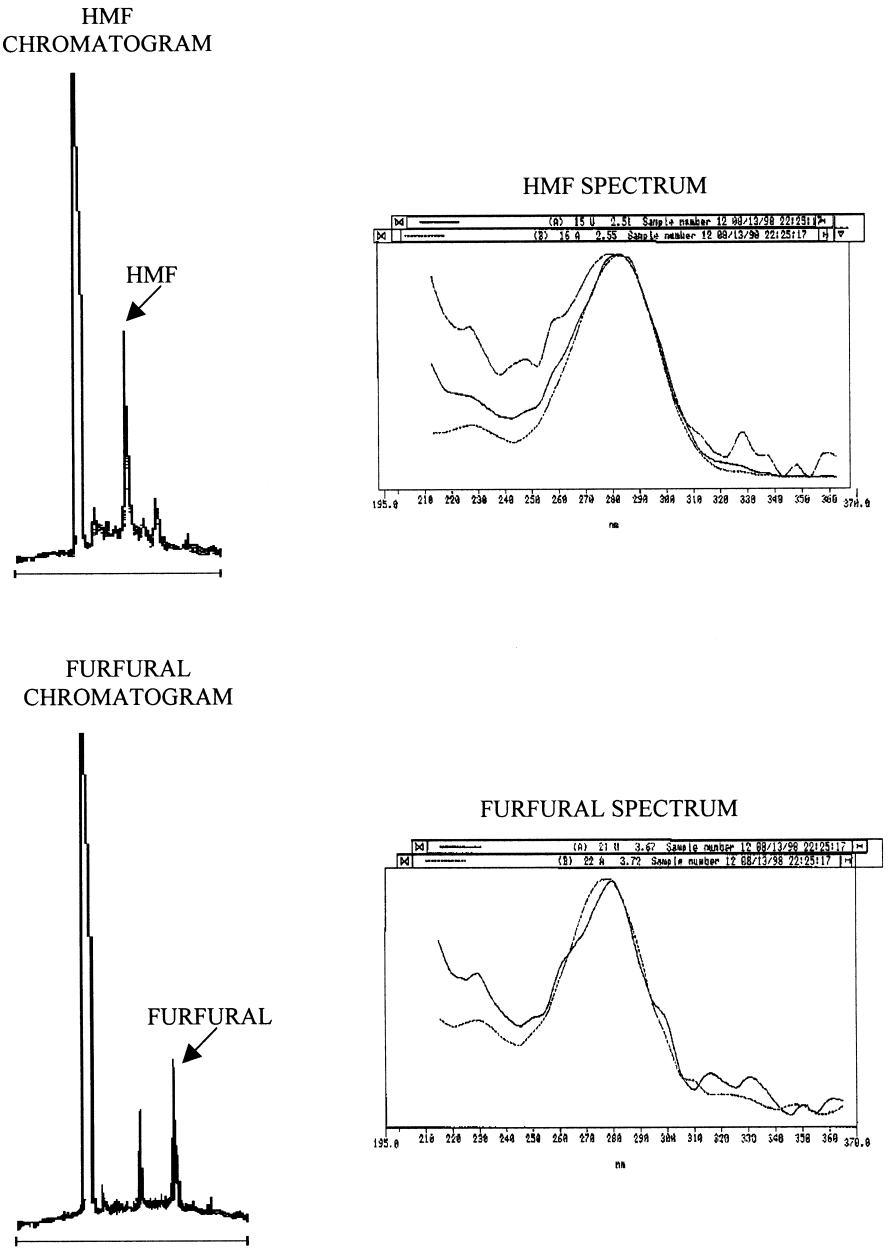

Figure 2. Spectra and chromatograms of purified furanic compounds of "R" enteral formula.

Table 1. HMF (mg/L) Recovery in the Analysis of Enteral Formula

Added	Total	Detected	Recovered (%)
0.16	1.19	1.16	97.5
0.41	1.44	1.47	102.1
0.74	1.77	1.82	102.9
0.82	1.85	1.82	98.3
1.96	2.99	2.99	95.5

n = 3.
Mean = 99.2 %.

ered with 1 mL ranged from 95.5 to 102.9% (Table 1) with an average value of 99.2%. The furfural recovered with 1 mL ranged from 73.3 to 70.0% (Table 2) with an average value of 71.1%. Three determinations were carried out for each addition level. The percentage of recovery was used to quantify the furfural in the samples.

In order to simplify the HMF and furfural determination, we added five increasing amounts of HMF and furfural of between 0.5-4.2 mg/L and 0.15-0.6 mg/L, respectively, to a sample of known concentration. Three purification steps with trichloromethane were performed, obtaining a mean recovery of 55.2% (C.V=1.26%) and 50.7% (C.V.=4.9%) for HMF and furfural, respectively. If only three purification steps are used, the percentage recovery must be applied in order to quantify the HMF and furfural in samples.

Precision

The study for each furanic compound was carried out on eight samples of enteral formula. The precision value for HMF was 2.41% (C.V.) for a mean value of 0.82 mg/L (formula P). For furfural, the precision value was 1.23% (C.V) for a mean value of 0.24 mg/L (formula R).

Table 2. Furfural (mg/L) Recovery in the Analysis of Enteral Formula

Added	Total	Detected	Recovered (%)
0015	0.015	0.011	73.3
0.030	0.030	0.021	70.0
0.050	0.050	0.036	72.0
0.070	0.070	0.049	70.0
0.100	0.100	0.070	70.0

n = 3.
Mean = 71.1 %.

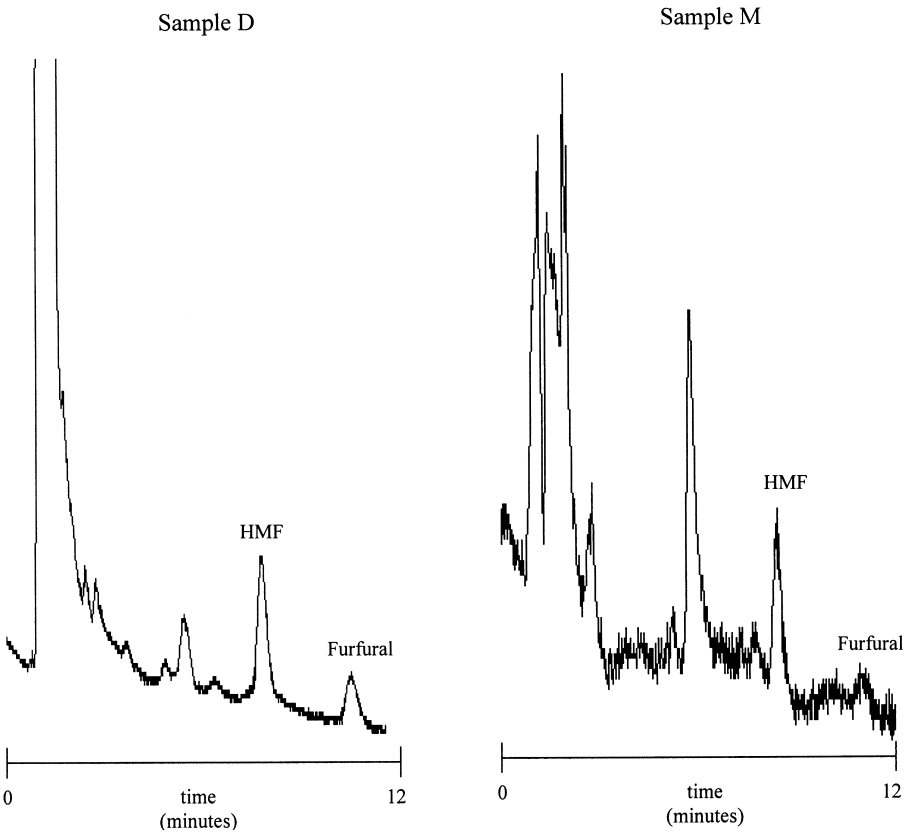

Figure 3. Chromatograms of low levels of furanic compounds.

Detection Limits

The detection limit was 0.01 mg/L for both HMF and furfural (calculated as signal-to-noise ratio of two). The quantification was performed on concentrations above 0.05 mg/L. Figure 3 shows the chromatograms of samples with low levels of furanic compounds.

Analysis of Samples

The method was applied to 18 commercial enteral formulas with different ingredients. The formulas were supplied by a local hospital and met the usual

Table 3. HMF and Furfural Content of Commercial Enteral Formula

Sample	Proteins (g/100 mL)		Carbohydrates (g/100 mL)		HMF (mg/L)	Furfural (mg/L)
A	Caseinate	7.1	Dextrinomaltose	3.6	19.1	0.58
	Whey proteins	1.6	Lactose	2.7		
			Sucrose	2.0		
B	Caseinate	4.2	Dextrinomaltose	7.8	2.10	n.d.[1]
			Fructose	1.8		
C	Milk proteins	9.7	Lactose	4.4	0.30	0.30
			Sucrose	4.2		
			Dextrinomaltose	1.8		
D	Caseinate	4.0	Dextrinomaltose	12.0	0.38	0.14
			Others	0.2		
E	Hydrolyzed soya proteins	4.7	Hydrolyzed corn starch	15.6	1.42	0.28
			Sucrose	4.9		
	Caseinate	1.3				
F	Hydrolyzed soya and lactoalbumin proteins	2.6	Hydrolyzed corn starch	12.6	0.52	0.72
			Sucrose	2.0		
			Fructose	1.0		
	Free aminoacids	2.6				
	Whey proteins	0.7				
G	Hydrolyzed lactoalbumin	3.5	Polysaccharides	10.9	0.154	0.38
			Sucrose	5.5		
			Dextrose	4.6		
H	Milk proteins	3.6	Dextrinomaltose fructose	7.0	1.00	n.q.[2]
I	Whey proteins	4.0	Hydrolyzed corn starch	20.7	0.78	0.28
			Sucrose	6.5		
J	Caseinate	4.1	Hydrolyzed corn starch	15.3	0.23	n.d.
	Whey proteins	1.4	Sucrose	4.8		
	Soya proteins	0.8				
K	Caseinate	4.1	Dextrinomaltose	11.7	1.00	n.q.
			Sucrose	2.7		
L	Caseinate	5.2	Hydrolyzed corn starch	18.5	0.05	n.d.
	Whey proteins	1.7	Sucrose	2.2		
			Fructooligosaccharides	1.6		
M	Milk proteins	2.8	Hydrolyzed corn starch sucrose	11.2	0.08	n.q.
N	Milk proteins	6.3	Dextrinomaltose Sucrose lactose	15.2	0.11	n.d.
O	Milk proteins	3.6	Dextrinomaltose	13.4	1.33	n.d.
P	Milk proteins	5.4	Dextrinomaltose	11.8	0.82	n.d.
			Sucrose	1.2		

Table 3. Continued

Sample	Proteins (g/100 mL)		Carbohydrates (g/100 mL)		HMF (mg/L)	Furfural (mg/L)
Q	Milk proteins	5.4	Dextrinomaltose	11.8	1.03	n.d.
			Sucrose	1.2		
R	Milk proteins	5.4	Dextrinomaltose	11.8	0.13	0.24
			Sucrose	1.2		

[1] Not detected.
[2] Not quantified.

nutritional needs of the majority of patients requiring nutritional support. Table 3 displays the concentrations of furanic compounds in the formulas. Although HMF values ranged from 0.05 to 19.1 mg/L, 66% of samples showed HMF concentrations of below 1 mg/L and 28% showed concentrations of between 1-2 mg/L. In nine samples, no furfural was detected. Furfural concentrations ranged from 0.14 to 0.72 mg/L.

Formula processing usually comprises four steps: ingredient blending, preheating (indirect-UHT or pasteurization), homogenizing, and sterilizing. HMF and furfural are produced in other foods by UHT and in-bottled sterilization treatments, with a correlation between the intensity of the heat and the level of their production.(19,20,21).

Model systems with casein-lactose and dextrinomaltose-casein were studied under adverse conditions of sterilization (120°C for 9, 15, and 30 minutes) and storage (55°C for 1 month). A preliminary study of HMF and furfural in the above ingredients revealed only HMF in the dextrinomaltose.

After heating the casein-lactose system for 9, 15, and 30 minutes at 120°C, the HMF content was 0.21, 0.35, and 0.69 mg/L, and the furfural content 0.04, 0.06, and 0.1 mg/L, respectively.

The same heat treatments of the dextrinomaltose-casein system produced no changes in HMF concentrations, and no furfural was detected.

After storage at 55°C for one month, the casein-lactose system showed HMF and furfural concentrations of 0.66 mg/L and 0.48 mg/L, and the casein-dextrinomaltose system showed an increase in concentration of 0.44 mg/L and 0.3 mg/L, respectively.

CONCLUSION

Our method to determine HMF and furfural in enteral formulas is precise and accurate, and includes an organic purification step, which is essential for

accurate determinations. The method could be simplified by the combined determination of HMF and furfural, using three steps of purification with trichloromethane and taking into account the percentage of recovery.

HMF can be used to control the ingredients enclosed in enteral formulas, and both HMF and furfural can be used to control the heat treatment of enteral formulas, especially when lactose is an ingredient.

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