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Postcolumn Derivatization Method for Determination of Reducing and Phosphorylated Sugars in Chicken by High Performance Liquid Chromatography

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A postcolumn derivatization method is described for determination of reducing sugars and phosphorylated reducing sugars from chicken meat and other foods using high-performance liquid chromatography (HPLC). Reducing sugars are extracted with ethanol/water, separated on a Kromasil amine-bonded column by isocratic analysis using acetonitrile/water as the mobile phase, and, after postcolumn reaction with tetrazolium blue, are determined by the resulting absorbance at 550 nm. Phosphorylated sugars are first dephosphorylated using alkaline phosphatase and then determined by the same method.

KEYWORDS: Postcolumn derivatization; phosphorylated sugars; reducing sugars; chicken; HPLC

INTRODUCTION

Reducing sugars are important for the flavor of cooked meats as they react with amino acids by the Maillard reaction during cooking to give many important flavor compounds (*1*). The addition of small quantities of ribose to raw beef has been shown to increase the quantities of key odor compounds, as well as meaty and roasted notes, in the cooked meat (*2*, *3*). Ribose-5-phosphate also caused important changes, whereas glucose and glucose-6-phosphate caused much smaller effects (*3*). In an aqueous model system, ribose-5-phosphate appears to be more reactive than ribose, producing much larger quantities of most volatile compounds responsible for meat flavor (*4*). To determine the relative importance of these reducing sugars for flavor formation in meat, it is necessary, first, to know their natural concentrations.

Although the concentrations of some sugars and sugar phosphates in meat have been determined previously (*5*−*7*), the reported concentrations show considerable variation. For example, the concentration of ribose in beef is reported as 1 mg 100 g^{-1} wet weight (*6*), 126 mg 100 g^{-1} wet weight (*8*) and 524 mg 100 g^{-1} wet weight (*9*). It is unclear whether this variation is due to differences between meat samples or analytical methods. To our knowledge, the natural quantity of ribose-5-phosphate in meat has not been reported.

Free sugars (glucose, fructose, and ribose) and sugar phosphates (glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate) have previously been identified and quantified in aqueous beef extract using gas chromatography with complex derivatization procedures (*6*). Glucose and glucose-6-phosphate have also been quantified in beef by enzymic methods (*7*). Concurrent analysis of reducing sugars and their phosphates is possible by anion exchange chromatography with pulsed amperometric detection using an alkali-resistant HPLC (*10*), and this method has considerable potential for rapid analysis times and good sensitivity, but the high cost of this system makes it unavailable in many laboratories.

The method described herein is based upon an HPLC method employed for the determination of reducing sugars in food and plant material (*11*). It utilizes the reducing power of the sugars for postcolumn derivatization with tetrazolium blue. An enzymatic reaction with alkaline phosphatase has been used to dephosphorylate sugar phosphates prior to analysis. Although this method has been used in our laboratory primarily for determination of reducing sugars and their phosphorylated homologues in chicken meat, preliminary results presented here indicate that it is also appropriate for other foods, such as beef, potato, and onion.

EXPERIMENTAL PROCEDURES

Materials. Analytical-grade chloroform (Lab-Scan, Dublin, Ireland), HPLC-grade ethanol (Hayman, Essex, UK), HPLC-grade acetonitrile, potassium tartrate, glycine, and magnesium chloride 6-hydrate (BDH Laboratory, Poole, UK), and HPLC-grade water (Aldrich, Poole, UK) were purchased and used without further purification. All reducing and phosphorylated sugars, (D-ribose-5-phosphate disodium salt, D-glucose-6-phosphate disodium salt hydrate, alpha-D-lactose monohydrate, D-ribose, D-glucose, D-fructose, D-fructose-1,6-diphosphate sodium salt, D-fructose-6-phosphate disodium salt), and tetrazolium blue (3,3′-[3,3′-dimethoxy(1,1′-biphenyl)-4,4′-diyl]-bis[2,5-diphenyl-2H-tetrazolium]-dichloride) were purchased from Sigma (Poole, UK). Alkaline phosphatase (phosphoric monoester phosphohydrolase, EC 3.1.3.1) *type VII-T*, from bovine intestinal mucosa, was also purchased from Sigma. The resins, Dowex 50WX4 (strongly acidic cation, 200−400 dry mesh) and

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Table 1. Recovery from Chicken and Detection Limit in Aqueous Solution

	% recovery								
sugar	(1)[a]	(2)	(3)	(4)	(5)	(6)	mean	SD	detection[b] limit
ribose	90	90	90	89	90	90	89.8	0.41	8.22×10^{-8} mol
glucose	94	97	98	94	84	91	93.0	5.06	9.63×10^{-8} mol
fructose	89	90	92	90	96	98	92.5	3.67	9.50×10^{-8} mol
R-5-P[c]	85	82	89	85	85	80	84.3	3.08	
G-6-P[d]	56	58	61	70	70	70	64.2	6.59	

[a] Values are the results of individual analysis. [b] Detection limit, in 20 μL injection of aqueous solution. [c] R-5-P = Ribose-5-phosphate. [d] G-6-P = Glucose-6-phosphate.

Anion WGR-2 (weakly basic anion, 20−4 wet mesh), and syringe filters (Acrodisc LC PVDF, 13 mm diameter, 0.2 μm pore size) were purchased from Aldrich.

Twenty-four chickens (from five commercial brands: A, B, and C (6 each); and D and E (3 each)) were purchased from local supermarkets, on separate occasions, to ensure that chickens were from several batches. The left breast (*pectoralis major*) and left leg (thigh and drumstick, combined muscles) were cut from each carcass, skinned, trimmed of any visible fat, homogenized separately, and prepared for extraction as soon as possible (within 30 min). All chickens were analyzed within the "use-by" date.

Extraction of Sugars. The extraction procedure used to isolate sugars from raw chicken was adapted from that of Jones (*12*), with the modifications made by Mandeville et al. (*13*). Approximately 50 g of raw chicken meat (breast or leg) was homogenized in a food processor (Robot Chef 2, Robot-Coupe, Vincennes, France). Duplicate samples (3 g) of the homogenized chicken meat were placed in 50-mL centrifuge tubes (Apex, Alpha Laboratories, Eastleigh, UK), and 0.5 mL of 40 mM α-D-lactose was added as an internal standard. The samples were homogenized with 10 mL of absolute ethanol for 3 min at full speed (Janke Kirka and Kunkle Werk, Labortechnik, Staufen, Germany), followed by centrifugation at 800*g* for 5 min (Heraeus Megafuge 1.0, Kalkberg, Germany). Extraction and centrifugation were repeated a further three times using 10 mL of 80% aqueous ethanol. The total volume of the combined supernatants was approximately 40 mL, to which 150 mL of chloroform was added. A separating funnel was used to extract any lipid and separate the phases; after approximately 40 min, the upper, aqueous phase was removed, and the organic phase was discarded. An aliquot (1.5 mL) of the extract (ca. 7.5 mL) was used for the analysis of phosphorylated sugars by enzymatic treatment.

The remaining aqueous phase (approximately 6 mL) was shaken for 3 min with 3 g of a mixture of 1:1 (w/w) Dowex 50WX4 and Anion WGR-2, to remove any interfering compounds, such as amino acids and salts. The resins were removed by centrifugation for 30 min at 800*g*, and the supernatant was transferred to HPLC vials and stored at 4 °C before use within the next 24 h.

Enzymatic Treatment. A solution of enzyme, alkaline phosphatase (0.55 units mL^{-1}), was prepared in glycine buffer (50 mM, containing $MgCl_2$ 0.5 mM, and adjusted to pH 9.3 using 1 N NaOH). To 3.5 mL of this solution, 1.5 mL of the meat extract was added. The solution was incubated in a water bath for 90 min at 39 °C. After the solution cooled to ambient temperature, it was filtered using an Acrodisc 13LC syringe filter to remove enzymes, and then treated with resins (3 g) as described above. Glucose phosphates and ribose phosphates were determined as glucose and ribose by difference between the total glucose and ribose with and without enzymatic treatment.

High-Performance Liquid Chromatography. The HPLC method was adapted from that of Wight and van Niekerk (*11*), and our apparatus was very similar to the schematic diagram shown in their paper.

The extracts were analyzed on an HPLC system equipped with a PC 1000 data system and a variable wavelength UV detector, Spectra System UV1000 (all from Thermo-Separation Products, Manchester, UK). An aliquot (20 μL) was injected for HPLC analysis. The sugars were separated on a Kromasil-NH_2 HPLC column (5-μ particle size, 100A pore size, 250 mm × 4 mm i.d.) from Phenomenex (Manchester, UK). A guard column with Kromasil-NH_2 cartridge was used (4 mm × 3 mm), also from Phenomenex. The isocratic mobile phase, acetonitrile/water (70:30) at pH 4.8, was degassed using helium for 40 min prior to chromatography.

Conditions for the postcolumn reaction were optimized with regard to reproducibility of determinations, speed of analysis, and sensitivity of detection. The reagent for postcolumn derivatization, tetrazolium blue (0.7 mM), was prepared in a solution of NaOH (0.16 M), ethanol (15%), and sodium potassium tartrate (0.047 M), in distilled water, pH 12.7. This solution was introduced by way of a three-port HPLC connection, at a flow rate of 1.8 mL min^{-1} using a secondary pump (model 510, Millipore-Waters, Milford, MA). The eluent and reagent passed through a coil of stainless steel tubing (5 m length, 3 mm i.d.), of which the front 4 m was heated in a water bath at 95 °C to facilitate the reduction reaction. The remaining 1m of stainless steel coil was held at ambient air temperature to cool the eluent prior to detection. The temperature of the eluent on reaching the detector was 25 °C. The total volume of the reaction tube was 0.8 mL.

Determination of Recovery, Reproducibility, and Limit of Detection. Recovery of each sugar from the raw chicken meat was determined by analyzing portions (3 g) of homogenized chicken muscle. Portions were spiked with a known amount of ribose (2.11 mg), glucose (2.18 mg), ribose-5-phosphate (3.05 mg disodium salt), and glucose-6-phosphate (3.02 mg disodium salt) in 0.2 mL of water. Samples of the same homogenized chicken without added sugars were also analyzed. The recoveries of reducing sugars and phosphorylated sugars were obtained separately. Percentage recoveries were based on the difference between the total amount in the spiked samples versus that in the unspiked samples. Reproducibility was assessed by the determination of recovery for six individual samples.

The limits of detection for each sugar were assessed in aqueous solution only (as chicken extracts always contained higher than these quantities). The limit of detection was determined as that concentration of sugar which gave a peak height three times that of the background noise.

RESULTS AND DISCUSSION

Validation of Method. The recoveries of ribose, glucose, fructose, ribose-5-phosphate, and glucose-6-phosphate were, respectively, 90, 93, 93, 84, and 64%. **Table 1** shows the six replicate determinations, and the reproducibility is indicated by the standard deviations cited. Coefficients of variation ranged from 0.5 to 10.3%. Earlier researchers (*12, 13*) using a similar extraction method have not commented on recoveries or reproducibility. However, Wight and van Niekerk *(11)*, using a similar postcolumn derivatization method, but with a different extraction method (hot water) and substrate (molasses), reported 99.1 and 98.1% recoveries for fructose and glucose, respectively.

The limits of detection of the HPLC method were 12.3, 14.5, and 14.3 μg per injection for ribose, glucose, and fructose, respectively, in aqueous solutions (**Table 1**); these quantities correspond to concentrations of 0.62, 0.72. and 0.71 mg mL^{-1}. These values are much higher than those reported by Wight and van Niekerk (*11*), who reported detection limits of 11, 6, and 55 ng per injection for glucose, fructose, and lactose. The relatively high detection limits reported herein are dictated by

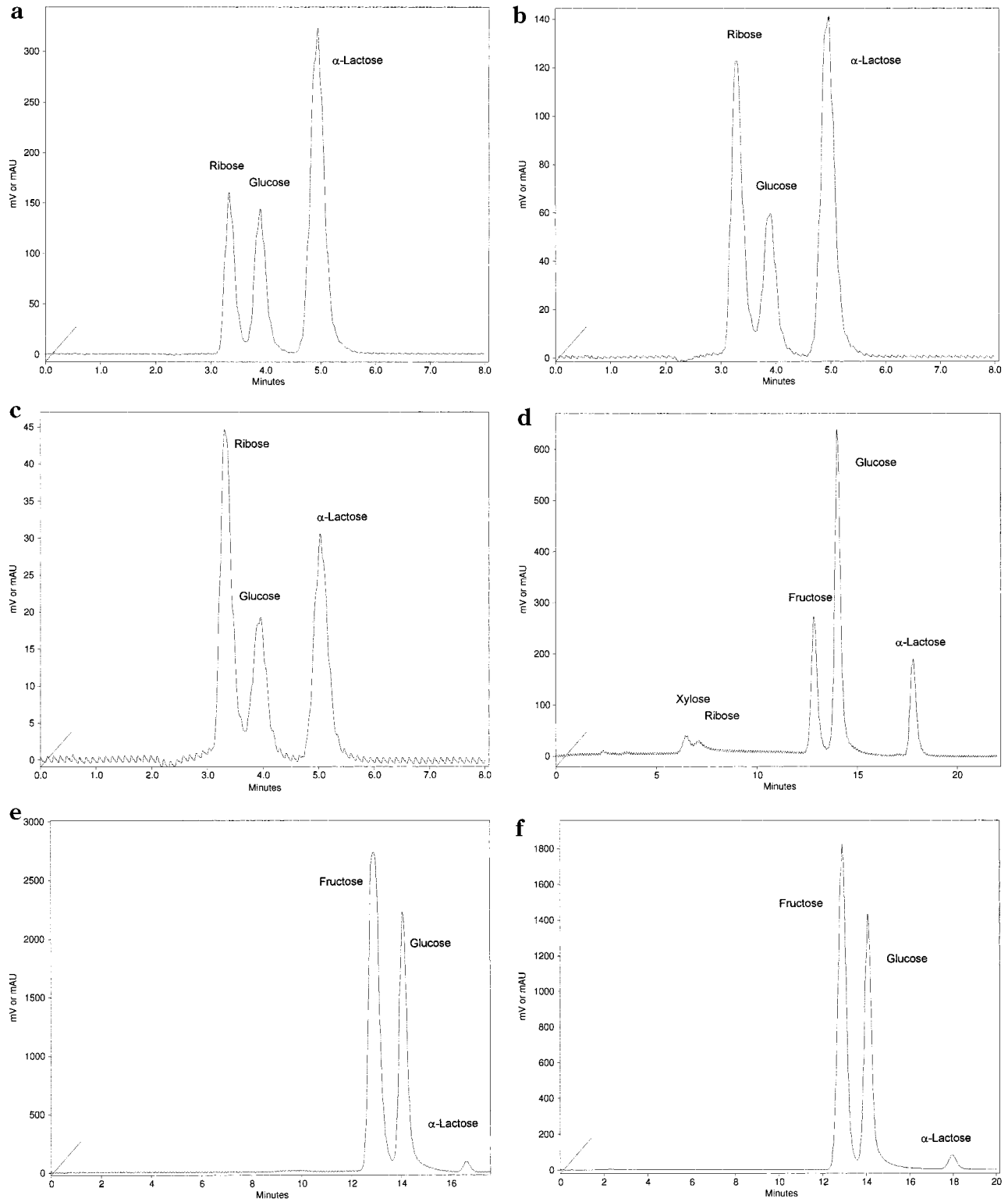

Figure 1. Typical examples of HPLC chromatograms, obtained as described and plotted to different scales, for (a) a standard solution of ribose, glucose, and α-D-lactose (120 μg, 140 μg, and 290 μg per injection), (b) chicken breast, without enzymatic treatment, (c) chicken breast after enzymatic treatment; and for (d) beef sirloin, (e) onion, and (f) potato, all without enzymatic treatment.

the amplitude of the systematic "noise" caused by the auxiliary pump used for the postcolumn reagent (**Figure 1c**). The detection limit was adequate for these studies, but could have been reduced by a factor of 100 by the use of a better pump.

Extraction Method. The extraction method using ethanol/water was chosen to extract mono- and disaccharides, but not proteins, while deactivating enzymes in the muscle tissue. Ethanol/water (80% v/v) has been reported to be a good general purpose extractant for monosaccharides in which proteins, polysaccharides, and many oligosaccharides are insoluble (*14*). Sugars may also be extracted with water (*15*−*17*), but this method was not used due to the risk of interference from other enzymes during the incubation of meat extract with alkaline phosphatase.

The addition of resin removes phosphorylated sugars as well as amino acids and salts from the extract. Therefore, when

extracting phosphorylated sugars, the resin was added after the enzymatic treatment. The ethanol/water extraction method was also employed without the use of resins to quantify amino acids (Aliani and Farmer, unpublished data), as described by Mandeville (*13*).

Enzymatic Reaction with Alkaline Phosphatase. Alkaline phosphatase was used to dephosphorylate glucose phosphates and ribose phosphates to their corresponding reducing sugars. The reactivity of alkaline phosphatase was tested at different pH values: 8.3, 9.3, and 10.3. The maximum activity of alkaline phosphatase toward both ribose-5-phosphate and glucose-6-phosphate was obtained at pH 9.3. The concentrations of enzyme in the incubation solution necessary to convert ribose-5-phosphate (1.3 mg mL^{-1}) into ribose, and glucose-6-phosphate (1.2 mg mL^{-1}) into glucose were 2.7 units mL^{-1} and 9.6 units mL^{-1}, respectively. The incubation time necessary for ribose-5-phosphate was found to be only 60 min at 39 °C, whereas glucose-6-phosphate required 90 min. Therefore, an enzyme concentration of 9.625 units mL^{-1} was used for a period of 90 min at 39 °C.

The conversion of phosphorylated sugars to their respective sugars in aqueous solutions was 100% for ribose-5-phosphate and 86% for glucose-6-phosphate whether they were present individually, together, or as a mixture with fructose phosphates. The poorer conversion of glucose-6-phosphate explains, in part, the lower overall recovery for this compound (**Table 1**). Efforts to improve conversion, and thus recovery, by increasing incubation time or quantity of enzyme added proved unsuccessful. Alkaline phosphatase converted fructose-6-phosphate (97%) more readily than fructose-1,6-diphosphate (68%) in aqueous solution, when treated separately. However, when these two compounds were treated together only 70% of the total phosphorylated fructose was converted. Some batches of alkaline phosphatase demonstrated an ability to convert glucose-6-phosphate not only to glucose but also fructose (ca. 5%). Treatment of aqueous glucose with alkaline phosphatase under the same conditions (pH 9.3, 39 °C) gave 100% recovery of glucose with no extra fructose peak, demonstrating that this effect was not due to conversion of glucose to fructose by the Lobry de Bruijn Alberda van Ekenstein reaction. Therefore, the conversion of glucose-6-phosphate to fructose is believed to be caused by an enzyme impurity, and batches of enzyme were tested with aqueous glucose-6-phosphate prior to use.

It is important to note that the alkaline phosphatase is nonspecific and does not distinguish between different sugar phosphates, converting all to the "parent" sugar. Thus, results for chicken and other foods are quoted as ribose phosphates, glucose phosphates, etc.

The extraction of sugars and sugar phosphates from eight samples of chicken (usually four sets of duplicates) was easily accomplished within one working day.

High-Pressure Liquid Chromatography. An internal standard was sought which was a reducing sugar, did not coelute with any of the analytes, and did not occur naturally in meat. Cellobiose was tested first but was found to be partially degraded during the enzymatic procedure with alkaline phosphatase; α-D-lactose did not show this effect and was, therefore, chosen as internal standard.

The proportion of acetonitrile and water in the mobile phase was critical for obtaining maximum resolution and speed of analysis. For the chicken extracts, an eluent comprising acetonitrile/water (70:30) at 1.7 mL min^{-1} was optimal. However, in samples containing sugars with closer retention times, such as fructose, glucose, mannose, and galactose, a lower eluent flow rate (1.4 mL min^{-1}), combined with a higher proportion of acetonitrile (85%) in the mobile phase, was necessary to give baseline separation. It proved advantageous to use the two pumps of the binary system to mix the acetonitrile with water to give a consistent ratio, as even small changes in the mixing ratio can influence chromatographic separation (*18*).

Postcolumn Derivatization Reaction. The postcolumn derivatization method using tetrazolium blue takes advantage of the reducing character of the sugars analyzed. The concentrations of sodium hydroxide, ethanol, sodium potassium tartrate and tetrazolium blue proved to be critical to ensure that the reaction between sugars and tetrazolium blue gave the appropriate product. Attempts to use the concentrations of NaOH (0.31 M and 0.36 M) recommended by previous authors (*11*, *19*) proved unsuccessful. This is because tetrazolium blue can be reduced to give two forms: the diformazan which has a blue color, and monoformazan (red), as described by Gorog (*20*). Concentrations of sodium hydroxide in excess of 0.26 M were found to favor the formation of the blue diformazan rather than the desired red monoformazan. The optimum concentration of NaOH for maximum reproducibility was found to be 0.16 M.

Previous authors (*21*) have reported that heat is necessary to dissolve tetrazolium blue. In contrast, in our study this was found to be unnecessary. Indeed, preliminary heating favored the formation of the blue diformazan, which was not desired. Likewise, ultrafiltration, filtration, and ultrasonification were found to be unnecessary, in contrast to a report by Vratny (*19*). Tetrazolium blue is freely soluble in the mixture of water, ethanol, and sodium hydroxide that was used for the dye solution. It turns from a light yellow to a purple red color after the reduction reaction with sugars takes place. Concentrations of more than 15% ethanol in the dye solution caused a problem with the stability and reproducibility of chromatograms. Chong (*22*) reported that the addition of sodium potassium tartrate to basic solutions of tetrazolium blue greatly improved their efficacy as colorimetric reagents for reducing sugars; it increased the sensitivity and the resolution of peaks and decreased the reaction time. In our studies the addition of sodium potassium tartrate provided sharper peaks with less tailing. The water bath temperature necessary for optimal and reproducible color formation was found to be 95 °C. Previous workers have found that temperatures of 85 °C (*11*) and 80 °C (*19*) were adequate, but in this study these temperatures gave poor reproducibility.

All monosaccharides, including sugar amines (glucosamine and galactosamine), are reducing sugars, which reduce tetrazolium blue, and can, therefore, be determined with this postcolumn derivatization method. The retention time of glucosamine and galactosamine are later than those of their parent sugars and they can be extracted as described, and the enzymatic reaction does not affect them. Reducing disaccharides, such as cellobiose, maltose, and lactose are also detected, but nonreducing sugars cannot be determined directly. Identification of each sugar was based on comparison of HPLC retention times with those of authentic sugars.

The HPLC method is extremely rapid; monosaccharides (ribose and glucose) and lactose can be analyzed in less than 7 min. For analyses of sugars in beef, the larger proportion of acetonitrile required in the mobile phase meant that the total analysis time was rather longer (20 min). A column life of over 10 months may be obtained, and the guard column had to be replaced at a frequency of about once a month.

Determination of Sugars and Sugar Phosphates In Chicken muscle. The method has been successfully applied to the analysis of reducing sugars and their phosphates in chicken meat.

Table 2. Concentrations (mg 100 g^{-1} wet weight) of Reducing and Phosphorylated Sugars in Breast and Leg of Chickens from Five Commercial Suppliers (A–E)

chicken number	breast					leg				
	ribose	glucose	RP[a]	GP[b]	total	ribose	glucose	RP[a]	GP[b]	total
A1	28.9[c]	35.3	8.0	10.7	83.0	14.1	11.7	7.0	8.6	41.4
A2	21.8	47.0	7.8	16.6	93.2	7.7	7.0	9.2	10.4	34.2
A3	28.5	28.5	10.0	11.1	78.1	10.3	4.4	5.3	9.2	29.2
A4	29.7	46.3	8.9	12.7	97.5	18.1	12.6	9.2	9.9	49.8
A5	28.3	37.1	10.9	12.2	88.4	17.2	17.0	5.4	9.9	49.5
A6	30.1	62.3	10.7	61.1	164.2	17.7	18.1	5.7	10.8	52.3
mean	27.9	42.7	9.4	20.7	100.7	14.2	11.8	7.0	9.8	42.7
SD	3.1	11.9	1.3	19.9	31.8	4.3	5.4	1.9	0.8	9.4
B1	7.1[b]	44.3	13.0	9.5	73.9	6.4	19.2	18.3	6.0	49.9
B2	18.8	38.1	19.5	9.6	86.0	11.4	14.3	18.9	5.7	50.3
B3	12.2	27.3	18.8	5.5	63.8	7.1	16.7	5.5	4.8	34.0
B4	22.5	34.8	13.5	6.5	77.3	12.4	20.9	15.6	10.6	59.5
B5	30.4	20.1	20.0	2.9	73.4	20.6	7.9	12.7	7.3	48.5
B6	19.4	28.1	3.0	8.0	58.5	11.5	10.1	4.9	4.9	31.4
mean	18.4	32.1	14.6	7.0	72.2	11.6	14.8	12.7	6.5	45.6
SD	8.1	8.7	6.5	2.6	9.8	5.1	5.1	6.2	2.2	10.7
C1	10.8	37.9	26.0	15.8	90.5	11.6	23.3	23.5	6.0	64.5
C2	15.1	11.6	28.5	11.5	66.7	11.7	6.6	16.4	8.3	43.0
C3	31.9	42.6	20.3	10.0	104.9	10.7	15.5	13.7	4.5	44.4
C4	29.4	37.6	26.3	18.1	111.4	16.6	18.7	24.0	15.7	75.0
C5	26.0	23.2	14.1	6.9	70.3	13.6	11.7	10.8	9.9	46.0
C6	12.6	30.3	6.9	19.5	69.3	11.3	16.8	6.9	6.2	41.2
mean	21.0	30.5	20.4	13.6	85.5	12.6	15.4	15.9	8.4	52.3
SD	9.2	11.5	8.4	4.9	19.6	2.2	5.8	6.9	4.0	14.0
D1	33.4	37.7	10.3	21.9	103.3	17.3	11.4	7.4	10.8	47.0
D2	26.3	62.1	8.7	35.0	132.0	17.8	16.4	8.4	10.1	52.6
D3	32.0	53.2	8.6	31.9	125.6	12.9	10.6	7.4	10.7	10.7
mean	30.5	51.0	9.2	29.6	120.3	16.0	12.8	7.7	10.5	36.7
SD	3.7	12.3	1.0	6.8	15.1	2.7	3.1	0.6	0.4	22.8
E1	24.0	52.0	12.0	16.0	104.0	17.7	22.0	16.7	9.0	65.4
E2	39.5	32.5	16.2	10.4	98.6	18.9	15.3	7.5	11.1	52.8
E3	33.4	100.4	7.6	43.2	184.6	24.6	90.2	7.7	16.5	138.9
mean	32.3	61.6	11.9	23.2	129.1	20.4	42.5	10.6	12.2	85.7
SD	7.8	35.0	4.3	17.6	48.2	3.7	41.4	5.3	3.9	46.5
mean (24)	24.7	40.4	13.7	16.9	95.8	14.1	17.4	11.2	9.0	50.5
SD	8.4	17.7	6.8	13.5	30.8	4.5	16.3	5.9	3.1	23.0
CV	33.9	43.8	49.5	79.7	32.2	32.0	93.4	52.4	34.1	45.6

[a,b] RP = ribose phosphates and GP = glucose phosphates. [c] Values are means of duplicate analyses.

Figure 1(a) illustrates a typical chromatogram of sugars in aqueous solution. The sugars, ribose (120 μg) and glucose (140 μg) elute first followed by the internal standard, α-D-lactose (290 μg). **Figure 1(b)** shows a typical chromatogram of sugars in chicken, without enzymatic treatment. Figure 1(c) shows the chromatogram of the same sample after enzymatic treatment. The decrease in peak heights between **Figures 1(b) and 1(c**) is due to the dilution effect of the enzyme/buffer solution.

The concentrations of reducing sugars (ribose and glucose) and phosphorylated sugars (ribose phosphate and glucose phosphate) in raw chicken breast and leg of 24 chickens from commercial sources are listed in **Table 2**. Results are the means of duplicate analyses. The average quantities of glucose and glucose phosphates in chicken muscle are 40.4 and 16.9 mg 100 g^{-1} in breast and 17.4 and 9.0 mg 100 g^{-1} in leg, respectively. The average concentration of ribose was determined to be 24.7 mg 100 g^{-1} in breast and 14.1 mg 100 g^{-1} in leg, whereas ribose phosphates were present at mean concentrations of 13.7 and 11.2 mg 100 g^{-1} in breast and leg.

Very few studies on determination of sugars in chicken have been reported. One comprehensive investigation of sugars (but not their phosphates) was conducted by Lilyblade and Peterson (*5*), using paper chromatography. These authors reported that glucose was the principal free sugar in chicken meat. This agrees with our findings for breast meat, but not always those for leg muscle. Based on the analysis of 2 chickens (13 weeks old), after 24 h chilled storage at 1 °C, they reported glucose concentrations of 236 mg 100 g^{-1} breast muscle and 139 mg 100 g^{-1} leg muscle (dry weight), corresponding to approximately 59 mg 100 g^{-1} and 35 mg 100 g^{-1} wet weight. These data are a little higher than those reported herein, probably due to the low temperature and short time of storage after slaughter. Lilyblade and Peterson (*5*) reported concentrations of ribose (dry weight) in freshly killed breast and leg muscle of 3 mg 100 g^{-1} and 1 mg 100 g^{-1}, respectively, which had increased to 5 mg 100 g^{-1} and 4 mg 100 g^{-1}, respectively, after 24 h storage at 1 °C. These amounts had increased to 14 mg 100 g^{-1} (breast) and 9 mg 100 g^{-1} (leg) after 6 days storage at 1 °C. These values correspond to 0.3 to 3.5 mg 100 g^{-1} wet weight and are very low compared with those determined in the current study; this may reflect the limitations of the analytical methods used at this time. These authors also reported the presence of fructose, 22 mg 100 g^{-1} leg muscle and 44 mg 100 g^{-1} breast muscle (dry weight), corresponding to approximately 5.5 and 11 mg 100 g^{-1} wet weight. In our studies, fructose was not detected in the chicken samples listed in **Table 2**. However, during preliminary studies, occasional birds were found to contain measurable (1−5 mg 100 g^{-1} muscle) quantities of fructose and fructose phosphate in both breast and leg.

Table 3. Effect of Commercial Source and Muscle on the Concentrations of Sugars and Sugar Phosphates

	source			muscle			anova two factors with replication		
carbohydrate	A	B	C	breast	leg	SEM	effect of source	effect of muscle	interaction
ribose	21.0	15.0	16.8	22.4	12.8	2.412	0.051	< 0.001	0.342
ribose phosphate	8.2[a]	13.6[a,b]	18.1[b]	14.8	11.8	2.375	0.001	0.137	0.854
glucose	27.3	23.5	23.0	35.2	14.0	3.484	0.415	< 0.001	0.062
glucose phosphate	15.3	6.8	11.0	13.8	8.3	3.531	0.071	0.065	0.345

[a,b] Values sharing the same superscript are not significantly different by Fisher's LSD test ($P < 0.05$).

We are unaware of any previous reports of the concentrations of ribose phosphates in chicken or any other meat. Surprisingly, considering the ready availability of enzymatic kit methods, there also appears to be little information on the concentration of glucose phosphates in chicken.

Statistical analysis using two factor ANOVA to compare breast and leg meat for those three sources (A, B, and C) for which six chickens were analyzed, showed a highly significant difference between sources for ribose phosphates, and a very highly significant difference between muscles for ribose and glucose (**Table 3**). Thus, breast muscle contained significantly more of both ribose and glucose than leg muscle, and source C consistently contained more ribose phosphates than chickens from source A. However, for most sugars, greater differences were observed between individual chickens than between sources.

The considerable variation in the concentrations of sugars between individual chickens from the same source is illustrated by the ranges of values, standard deviations, and coefficients of variation in **Table 2**. For ribose, the coefficients of variation in breast and leg were ca. 33% with individual chickens having concentrations ranging from 7.1 to 39.5 mg 100 g^{-1} (breast) and from 6.4 to 24.6 mg 100 g^{-1}(leg). The coefficient of variation for ribose phosphates was higher, ca. 50% for both muscles. For glucose and glucose phosphates, the coefficient of variation was again high, but differed between the two muscles (**Table 2**). In many cases, the mean and standard deviation within a source are comparable with the overall values. This variation between individual chickens is probably due to natural genetic variation and may also be affected by differences in time after slaughter in commercially available chickens. Lilyblade and Peterson (*5*) reported that ribose was present only in trace amounts in freshly killed chicken, but increased in all samples during storage. Muramoto (*23*) reported that the content of ribose increased in fish after 48 h at 4 °C. Jones (*12*) reported that in codling, as spoilage progresses, the proportion of the two sugars (ribose and glucose) changes. The effect of time post-slaughter on concentrations of these sugars in chicken is the subject of ongoing studies.

Application of Method to Other Foods. The method described has also been applied to the analysis of sugars and their phosphates in beef and has been tested for the analysis of sugars in onion and potato.

Figure 1(d) shows a chromatogram of sugars extracted from beef sirloin. Glucose, fructose, and ribose were found to be the major carbohydrates together with glucose and fructose phosphates. Our results agree with the findings of Tonsbeek et al. (*24*) and Jarboe and Mabrouk (*6*) who found these sugars and sugar phosphates in beef. Preliminary results, based on the analysis of 10 samples of sirloin (Farmer and Hagan, unpublished data), suggest that beef contains approximately 148, 33, and 26 mg 100 g^{-1} (wet weight) of glucose, fructose, and ribose, together with 80, 32, and 9 mg 100 g^{-1} of the corresponding phosphates.

Figures 1(e) and **(f)** show the chromatograms of sugars in extracts of onion and potato. The major reducing sugars detected in onion were fructose, glucose, fructose phosphates, and glucose phosphates. The quantities of sugars were much greater than those detected in meat, and are estimated to be the order of 0.5 to 1.5 g 100 g^{-1} wet weight. The same sugars were detected in potato, at concentrations of approximately 1 g glucose and 0.4 g fructose 100 g^{-1} potato (wet weight), with lower concentrations of the phosphorylated sugars. Thus, the method reported in this paper provides a relatively straightforward and robust method for the analysis of these reducing sugars and their phosphates in a range of foods.

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