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Effect of carbohydrate DE on blocked lysine and furosine in a liquid nutritional product

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ABSTRACT

A controlled, quantitative study of Maillard browning vs. dextrose equivalents (DE) was performed on a liquid nutritional product. The early stage Maillard markers furosine and available lysine were determined in retort-sterilised, pilot scale batches formulated with carbohydrate systems with DE variations of 2, 4, 10, and 20. Both markers varied proportionately with DE; every DE increase of 2 units resulted in the blockage (glycation) of an additional 1% of total lysine. When DE 20 maltodextrin was replaced with an 80/20 blend of DE 5 maltodextrin and sucrose (blend DE = 4), lysine blockage decreased by 831 mg/100 g protein, which was 8.25% of the total lysine, and the mole equivalent of 1.75 g of fructoselysine. The quantitative browning/DE relationships enable reliable projections of the nutritional benefits which may be attained through the use of low-DE maltodextrins.

1. Introduction

A review of Maillard chemistry recommended the prevention of browning and its negative impact on foods and feeds, where the prevention strategies should follow multidisciplinary studies defining the in vivo effects of potentially problematic Maillard reaction products (Friedman, 1996). Possible negative effects associated with browning include (a) a reduction in nutritional quality – via modification of essential amino acids, impaired digestibility, and/or restricted mineral availability – and (b) the generation of reaction products whose toxicity – mutagenicity, carcinogenicity, allergenicity, clastogenicity, and cell deactivation – has been asserted or suggested (Finot, 1990; Friedman, 1996; Friedman & Cuq, 1988; Gumbmann, Friedman, & Smith, 1993; Jing & Kitts, 2004; Knipfel, Botting, & McLaughlan, 1975; MacGregor et al., 1989; Seifert, Krause, Gloe, & Henle, 2004). A recent review of modified amino acid metabolism studies (Finot, 2005) associated measurable absorption and availability limitations even with early stage browning products such as Amadori compounds and protein-bound fructoselysine (Erbersdobler, Gunsser, & Weber, 1970; Rerat, Calmes, Vaissade, & Finot, 2002). Finot (2005), like Friedman (1996), cited the need for a closer connection between toxicological study conditions and the actual human consumption and metabolism of conventional processed foods. To the recommended end of preventing browning in formulated nutritional products, numerous researchers have noted that this may be at least partially attained through carbohydrate (CHO) ingredient selection, by exploiting differences in CHO "dextrose equivalents" (DE), where DE is a measure of the reducing sugar content of the CHO (Contreras-Calderon, Guerra-Hernandez, & Garcia-Villanova, 2008; Evangelisti, Calcagno, & Zunin, 1994; Knipfel et al., 1975; Rufian-Henares, Guerra-Hernandez, & Garcia-Villanova, 2006). Since a reducing sugar often comprises one of the two primary participants (the other participant generally being a primary or secondary amino compound) that initiate the Maillard reaction, a decrease in reducing sugar concentration effectively restricts the browning process. Accordingly, in the case of formulated nutritional products, a decrease in reducing sugar concentration may be achieved by favouring low-DE CHO ingredients; e.g., by substituting sucrose (a nonreducing sugar) for lactose (a reducing disaccharide), by substituting starch (a nonreducing polysaccharide) for corn syrup solids (DE = 20–36), or by substituting DE 5 maltodextrin for DE 20 maltodextrin. This concept has been incorporated into the design of the present study, a quantitative characterisation of Maillard browning across DE variations on a nutritional product formulation. Pilot scale batches of the nutritional product were prepared with CHO systems whose DE ranged from 2 to 20, and the early stage Maillard browning markers available lysine and furosine were determined after the (quadruplicate) production of each batch. The blocked (glycated) lysine concentrations were calculated from determinations of acid-released lysine, enzyme-released lysine, and furosine, where the enzyme hydrolysis procedure used the three-enzyme combination (pronase, leucine

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aminopeptidase, and prolidase) described by Henle, Walter, and Klostermeyer (1991). Furosine concentrations were determined in the same acid digest (6 M HCl) prepared for total lysine quantification. The blocked lysine and furosine concentrations were plotted vs. DE, so that the study constitutes a controlled and quantitative assessment of Maillard browning dependence on the DE of the nutritional product CHO system.

2. Materials and methods

2.1. Liquid nutritional product

The study was performed on pilot scale (20-kg) variations of a liquid nutritional product (LNP). The LNP contained partially hydrolysed milk protein at approximately 76 g per kg of LNP. The protein degree of hydrolysis (DH) was approximately 10%. LNP batches with four CHO system variations were prepared: Variable A was formulated with a blend of sucrose (60% of CHO) and DE 5 maltodextrin (40% of CHO), Variable B with a 20/80 blend of sucrose and DE 5 maltodextrin, Variable C with DE 10 maltodextrin (100% of CHO), and Variable D with DE 20 maltodextrin (100% of CHO), such that the DE values were 2.0, 4.0, 10, and 20, respectively, for LNP Variables A, B, C, and D. Included in all LNP batch variations was a nutritionally complete system of lipids, vitamins, and minerals, as well as a vanilla flavouring and an emulsion stabiliser. All batches were prepared in the Abbott Nutrition pilot plant (Columbus, OH, USA), were subjected to both UHT and retort sterilisation heat treatments, and were filled in 237-mL metal draw/redraw cans. All batches were tested for total lysine, available lysine, and furosine within five days of pilot plant manufacture.

2.2. Standards and reagents

Standard reference materials of L-lysine monohydrochloride (>99.5%) and furosine dihydrochloride were obtained from Fluka (Milwaukee, WI, USA) and from NeoMPS, Inc. (San Diego, CA, USA), respectively. The reagents boric acid, citric acid, 9-fluorenylmethyl chloroformate (FMOC-Cl), PIPES (piperazine-1,4-bis[2-ethanesulphonic acid]), potassium phosphate monobasic (KH_2PO_4), sodium azide, and sodium hydroxide, as well as the purified protein bovine serum albumin (BSA), were obtained from Sigma–Aldrich (St. Louis, MO, USA). Redistilled 6 M HCl was obtained from GFS Chemicals (Columbus, OH, USA). HPLC grade acetonitrile was obtained from Honeywell Burdick & Jackson (Muskegon, MI, USA). The proteolytic enzymes pronase E (EC 3.4.24.31; protease from *Streptomyces griseus*, type XIV, ~4 units/mg of solid, containing calcium acetate), leucine aminopeptidase, microsomal (EC 3.4.11.2; from porcine kidney, type VI-S, lyophilised powder, 15–25 units/mg of protein, containing primarily phosphate buffer salts), and prolidase (EC 3.4.13.9; from porcine kidney, lyophilised powder, salt-free, 100–300 units/mg of protein) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Analytical methods

2.3.1. Determination of furosine

Furosine concentrations were determined by acid hydrolysis/HPLC. A 0.2 g aliquot of LNP (i.e., ~15 mg of protein) was weighed into a 5-mL glass ampule (Wheaton, Millville, NJ, USA), and 5.00 mL of 6 M HCl were added. The ampule was nitrogen-blanketed, flame-sealed, and placed in an oven maintained at 110 °C for 22 h. After cooling to room temperature (RT), the ampule was opened, and the contents were quantitatively transferred into a 25-mL volumetric flask, using 0.05 M KH_2PO_4, pH 2.9, to assist with the transfer. The acid was neutralised by carefully adding 2.90 mL of 10 M NaOH to the 25-mL flask, and after cooling to room temperature (RT) (22 °C), the mixture was diluted to volume with 0.05 M KH_2PO_4, pH 2.9. This preparation was syringe filtered (Acrodisc PSF, 0.45 μm, PALL Life Sciences AP-4426) into an HPLC autosampler vial. The vial was sealed and loaded in the HPLC autosampler. Direct determination of furosine was performed on an Agilent model 1100 HPLC system (Agilent Technologies, Wilmington, DE, USA) with a model G1315B diode array detection (DAD) system (Agilent Technologies). The system was equipped with a 250 mm × 4.6 mm ID, 5 μm, YMC Hydrosphere C18 reversed-phase analytical HPLC column (Waters, Milford, MA, USA). The column was maintained at 15 °C during analyses by a model G1316A thermostatted column compartment (Agilent Technologies). The detection wavelengths were 280 nm (for furosine quantification) and 214 nm (for peak purity assessment only), with reference wavelength at 590 nm. The determination used a binary, step gradient elution with flow rate at 0.3 mL/min, and with vacuum-degassed mobile phases A (0.05 M KH_2PO_4, pH 2.9) and B (800/200, v/v, acetonitrile/Milli-Q Plus water). The injection volume was 3 μL, and the elution programme was 0% B from 0.0 to 10.0 min, 100% B from 10.1 to 20.0 min, and 0% B from 20.1 to 45.0 min. The system was calibrated by including in each analysis three furosine reference standard solutions, prepared at 0, ~10, and ~20 μM in 0.05 M HCl. System linearity was characterised as the coefficient of determination (r^2 = 0.99994) average, and the middle standard residual average (−0.6%), calculated for five furosine standard curves. Furosine peak purity was characterised as the 280 nm/214 nm peak area ratio, expressed as a % of the same ratio (approximately 11:1) calculated for the furosine peak in the standard solution injections. Experimental peak purity values for five independent determinations ranged from 97.1% to 108.9%, with an average of 102.7 ± 4.2%, n = 10. Furosine method precision was characterised as day-to-day RSD for duplicate determinations performed on five days; the experimental RSD value was 2.5%, n = 10.

2.3.2. Determination of total lysine

Total lysine concentrations were also determined by acid hydrolysis/HPLC, using the same acid digest described above for furosine determination. From the neutralised preparation diluted to volume in the 25-mL flask (prior to syringe filtration), 20.0 mL were transferred into a 50-mL beaker, a 20.0 mL of Milli-Q Plus water were added, and the pH was adjusted to 6.5–7.5 with 10 M NaOH. The mixture was quantitatively transferred into a 50-mL volumetric flask, using Milli-Q Plus water to assist with the transfer, and was diluted to volume with Milli-Q Plus water. In order to quantify the lysine in this preparation, a pre-column derivatisation with the fluorescent tag FMOC was used. This was accomplished as follows: (a) 100 μL was pipetted (from the 50-mL flask) into a 10-mL volumetric flask, (b) 500 μL of 0.40 M sodium borate, pH 8.5, were added, (c) 500 μL of FMOC-Cl prepared at 4 mg/mL in acetonitrile were added, (d) the flask was swirled gently to mix the reagents, (e) the flask was allowed to stand at RT (22 °C) for 5 min, and (f) the flask was diluted to volume with HPLC mobile phase A. An aliquot was transferred into an HPLC autosampler vial, which was then sealed and loaded into the autosampler for quantification of N_α,N_ε-di-FMOC-lysine. Also derivatised (in separate 10-mL flasks) at the same time, and by the same procedure, were (a) 100 μL of a standard blank (Milli-Q Plus water), (b) 100 μL of a 150 μM solution of L-lysine reference standard in Milli-Q Plus water, and (c) 100 μL of a 300 μL solution of L-lysine reference standard in Milli-Q Plus water. The derivatised standard and sample solutions (which were stable for 24 h at RT) were tested for N_α,N_ε-di-FMOC-lysine by an Agilent model 1100 HPLC system equipped with a model G1321A fluorescence detector (FLD, Agilent Technologies). The system was equipped with a 250 mm × 4.6 mm ID, 5 μm, 120 Å, YMC-Pack ODS-AQ reversed-

phase analytical HPLC column (Waters, Milford, MA, USA). The column was maintained at 40 °C during analyses by a model G1316A thermostatted column compartment (Agilent Technologies). The detection parameters were excitation wavelength = 262 nm, emission wavelength = 310 nm, and gain = 10. The determination used a binary gradient elution with flow rate at 0.5 mL/min, and with vacuum-degassed mobile phases A (650 mL 0.05 M sodium citrate, pH 3.0, +350 mL acetonitrile) and B (200 mL 0.05 M sodium citrate, pH 3.0, +800 mL acetonitrile). The injection volume was 4 μL, and the elution programme was 0% B from 0.0 to 16.0 min, 100% B from 16.1 to 50.0 min, and 0% B from 50.1 to 60.0 min. System linearity was characterised as the coefficient of determination (r^2 = 0.99996) average, and the middle standard residual average (+0.4%), calculated for five N_α,N_ε-di-FMOC-lysine standard curves. Method precision was characterised as day-to-day RSD for duplicate determinations performed on five days; the experimental RSD value was 1.7%, n = 10. Accuracy was assessed by quantifying (via triplicate determination) the recovery of a known addition of protein-bound lysine to the LNP. This was accomplished by (a) spiking the LNP with a reference lot of purified bovine serum albumin (BSA, Sigma–Aldrich, St. Louis, MO, USA), by (b) determining the total lysine concentration in unspiked and spiked samples of the LNP, and by (c) calculating the lysine spike recovery vs. the expected total lysine for the BSA reference material (expected lysine = 12.98 g/100 g of protein [UniProt Consortium]). The lysine recovery obtained by this experimentation was 99.8 ± 2.2%, n = 3.

2.3.3. Determination of available lysine

Available lysine concentrations were determined by enzyme hydrolysis/HPLC. A 0.4 g aliquot of LNP (i.e., ~30 mg of protein) was weighed into a 10-mL volumetric flask, and diluted to volume with 0.05 M PIPES, pH 7.5, containing sodium azide at 0.1% (w/w). To 1000 μL of this buffered sample suspension aliquoted into a glass autosampler vial (1.8 mL, 12 × 32 mm, VWR International, Bristol, CT, USA) were added 50 μL of pronase E (prepared at 9.2 units/mL, in pH 7.5 buffer), 20 μL of leucine aminopeptidase (prepared at 24 units/mL in Milli-Q Plus water), and 10 μL of prolidase (prepared at 180 units/mL in Milli-Q Plus water). The vial was crimp-sealed (PTFE/silicone/PTFE seal, VWR International), gently inverted to mix contents, and incubated at 37 °C for 20 h. An enzyme blank preparation (all three enzymes added to 1000 μL of buffer only) was included in each analysis to quantify the lysine contribution from enzyme autolysis. After the 20-h incubation, 500 μL of digest were diluted to 5 mL with Milli-Q Plus water. The free lysine (available lysine) in this preparation was then determined via the same FMOC derivatisation procedure, and by the same HPLC system, as described above for the determination of total lysine. Method precision and accuracy were characterised in the same manner as described above for the determination of total lysine. The experimental RSD value was 1.1%, n = 10, and the lysine recovery was 92.7 ± 1.4%, n = 3.

3. Results and discussion

Lysine and furosine concentrations determined in the LNP variables are shown in Table 1. DE values, as calculated from the LNP formulation (total CHO content) and from the DE values associated with the individual CHO ingredients, are specified in the same table. Since the LNP contains partially hydrolysed proteins, such that the lysine ε-amino group comprises only about 40% of the primary amino total, the mole ratios of DE/total lysine, and of DE/amino nitrogen, have been included. Blocked lysine, which is the available lysine subtracted from the total lysine, was expressed on a protein basis and on a total lysine basis. The values designated as "apparent blocked lysine" have been adjusted to exclude a portion of the total LNP lysine (7.31%) that seems to reside in native peptide linkages from which the analytical proteases cannot completely release free lysine (as opposed to lysine blocked by non-native glycation). The basis for the adjustment has been described below. The furosine concentrations were expressed in a similar manner, and were further expressed as % of blocked lysine. As the DE increases from 2.0 to 20, and as the mole ratio of DE/total lysine increases from 0.393 to 3.88, blocked lysine more than doubles (from 8.51% to 17.7%), and furosine increases nearly sevenfold (from 49.8 to 340 mg/100 g protein). A plot of blocked lysine (as % of total lysine) vs. DE illustrates the strong positive correlation (r^2 = 0.9934). The slope of the plot (0.5091) indicates that every 2-unit increase in DE will result in the blockage of another 1% of the total lysine. The y-intercept of the plot suggests that >7% of the total lysine was inaccessible to enzyme release even in the absence of dextrose (i.e., for DE = 0), presumably signifying the presence of lysine peptide linkages which cannot be completely cleaved to release free lysine by the proteases (pronase, leucine aminopeptidase, and prolidase) used in the enzyme hydrolysis (Baxter, Phillips, Dowlati, & Johns, 2004; Tsao & Otter, 1999). This presumption appears to be further supported by the lysine released (92.7 ± 1.4%, n = 3) from purified BSA in the experimental assessment of method accuracy, wherein an equivalent fraction of the total lysine (7.3%, on average) was not released by the enzyme hydrolysis. The strong positive correlation between furosine and DE can be seen in a plot of the data (r^2 = 0.9964), and here the slope projects that every 2-unit increase in DE will generate an additional 30–35 mg of furosine per 100 g of LNP protein. The y-intercept of this plot corresponds to a very low level of furosine (<11 mg/100 g protein, or less than 0.1% of the total mole lysine in the LNP), which would also seem to support the presumption that approximately 7% of the total lysine in the LNP protein ingredients is not glycated, but resides in peptide linkages inaccessible to cleavage by the proteases at issue. Additionally, it is worth noting that a plot of furosine mole % of blocked lysine vs. DE ($y = 0.4069x + 3.1055$; r^2 = 0.9807) exhibits a positive slope which also seems consistent with a constant presence of protease-inaccessible lysine in the LNP protein ingredients. If we proceed on the assumption that 7.31% (the y-intercept of the plot of blocked lysine vs. DE) of the LNP total lysine resides in peptide bonds that are inaccessible to complete cleavage by the analytical enzymes, then a plot of blocked lysine (or "apparent blocked lysine") vs. DE ($y = 0.5091x - 0.0017$; r^2 = 0.9934) clearly shows the near proportionality between "apparent blocked lysine" and DE; i.e., a doubling of the DE results in a near doubling of the apparent blocked lysine.

The DE-based correlations defined here are very much consistent with the findings of Evangelisti et al. (1994), drawn from their study of blocked lysine dependence on various CHO systems (most notably lactose and maltodextrins with DE ranging from 11 to 28). The Evangelisti study (1994) included thirty commercial spray-dried infant formulas, with mole ratios of DE/total lysine ranging from <0.5 to >2.0. The authors' observation that blocked lysine generally increased as this DE/lysine ratio increased led them to accurately predict the nutritional benefit quantitatively demonstrated in the present study: a significant increase in available lysine accompanied the use of low-DE maltodextrin. Further support was provided by the research of Knipfel et al. (1975), demonstrating less nutritional damage with nonreducing CHO (e.g., starch) than with reducing CHO (glucose), and by a recent study of infant formula powders made with combinations of lactose, sucrose, and/or maltodextrin (DE = 17), wherein blocked lysine decreases and furosine increases were associated with the substitution of maltodextrin for lactose (Contreras-Calderon et al., 2008). A model system comparison of available lysine after heating proteins in the presence of either lactose or maltodextrin (DE = 19) found higher recoveries with the latter, offering addi-

Table 1
Lysine and furosine concentrations vs. LNP variable (n = 4 batches/variable).

	Variable A	Variable B	Variable C	Variable D
DE[a] of LNP CHO	2.0	4.0	10	20
DE, as g/kg of LNP	3.65	7.30	18.3	36.5
DE, as mmoles/kg of LNP	20.3	40.6	102	203
Total lysine, mmoles/kg of LNP	51.7 ± 1.7	51.5 ± 0.0	53.1 ± 2.2	52.3 ± 1.5
DE/total lysine, mole	0.393	0.788	1.92	3.88
DE/amino N, mole	0.162	0.325	0.816	1.62
Blocked lysine, g/100 g protein	0.847 ± 0.071	0.949 ± 0.056	1.22 ± 0.12	1.78 ± 0.13
Blocked lysine, % of total lysine	8.51 ± 0.81	9.45 ± 0.54	11.9 ± 0.7	17.7 ± 1.2
"Apparent blocked lysine", %[b]	1.20	2.14	4.59	10.4
Furosine, mg/100 g protein	49.8 ± 2.1	74.7 ± 6.7	162 ± 22	340 ± 6
Furosine, mole % of total lysine	0.287 ± 0.016	0.472 ± 0.020	0.911 ± 0.095	1.94 ± 0.09
Furosine, mole % of blocked lysine	3.40 ± 0.37	5.02 ± 0.43	7.65 ± 0.52	11.0 ± 0.81

[a] DE = dextrose equivalents; all DE values were calculated from the LNP formulation.
[b] Based on the assumption that 7.31% of the total lysine is protease-inaccessible.

tional support (Rufian-Henares et al., 2006). In a study of 24 commercial liquid infant formulas whose CHO system was "essentially lactose", Birlouez-Aragon et al. (2004) reported blocked lysine averages of 20.7% for casein-dominant, and 25.2% for whey-dominant, products. The present study suggests that this magnitude of lysine blockage (~20–25%) could be substantially decreased in the liquid infant formulas by replacing the lactose with a low-DE maltodextrin.

Furosine has been widely used as an indicator of early stage Maillard reactions, so much so that (a) a factor – 3.1x – has been determined to estimate glycated lysine from furosine (Bujard & Finot, 1978), (b) processing-specific furosine concentrations (e.g., an upper limit of 250 mg/100 g protein for UHT treated milk products) have been suggested as quality control criteria (Clawin-Radecker & Schlimme, 1995), and (c) limitations associated with furosine-based projections have been defined (Henle et al., 1991; Claeys, Smout, Van Loey, & Hendrickx, 2004). It should be noted that whereas furosine is more often determined via a 7–8 M HCl hydrolysis (Leclere, Birlouez-Aragon, & Meli, 2002; Morales & Jimenez-Perez, 2000), the present study used a 6 M HCl hydrolysis. Accordingly, comparisons will be restricted to published data which were also obtained via 6 M HCl hydrolysis. Villamiel, Vazquez, Morais, and Corzo (2000) measured furosine at 23.7–67.2 mg/100 g protein in milk which was heated for 2 s at temperatures from 105 to 125 °C. Corzo, Delgado, Troyano, and Olano (1994) reported furosine at approximately 55–270 mg/100 g protein in UHT treated milk (31 batches), and at approximately 170–340 mg/100 g protein in commercial nonfat dry milk (11 batches). Baptista and Carvalho (2004) found furosine at 9.7 mg/100 g protein in raw milk, at 91 mg/100 g protein in evaporated milk, and from 21.4 to 81.5 mg/100 g protein in a selection of thirteen dietetic infant formula (DIF) powders. Using the conversion factor mentioned above, Baptista and Carvalho (2004) calculated blocked lysine at 7–26% for the same DIF powders. It is interesting to note that if blocked lysine is plotted vs. furosine for the LNP data of the present study, a straight line (r^2 = 0.9995) with slope = 3.18 is obtained, the value of the slope being nearly identical to the factor (3.1) first determined by Bujard and Finot (1978) and subsequently used by Baptista and Carvalho (2004). In a related but independent finding, we measured the increases of blocked lysine (1650–2080 mg/100 g protein) and of furosine (344–479 mg/100 g protein) that occurred between the production of an LNP variable (i.e., at 0-time) and after a six-month RT storage. Here again the ratio (w/w) of blocked lysine increase (430 mg/100 g protein) to furosine increase (135 mg/100 g protein) approximated 3.1 (actual ratio = 3.19). In both cases – blocked lysine/furosine vs. DE and blocked lysine/furosine vs. shelf life – the experimental ratio closely approximated the 3.1x factor reported thirty years ago (Bujard & Finot, 1978). Finally, Hurrell and Finot (1983) applied another furosine conversion factor (provided by Finot, Deutsch, & Bujard, 1981) to calculate "reactive lysine" (available lysine) concentrations in whole milk powder subjected to various storage conditions: reactive lysine = total lysine $-$ 1.24 $\times$ furosine. If this same calculation, but revised to subtract the y-intercept of the blocked lysine vs. furosine plot (701 mg/100 g protein), is applied to the LNP furosine data of the present study, then reactive lysine concentrations of 9.20, 9.13, 9.33, and 8.95 g/100 g of protein are obtained for Variables A, B, C, and D, respectively. All of these reactive lysine concentrations comfortably exceed published lysine requirements for both infants (6.6 g/100 g protein) and adults (5.0 g/100 g protein) (Young & Borgonha, 2000).

4. Conclusions

Blocked lysine and furosine were found to increase proportionately as the DE of a liquid nutritional product was increased from 2 to 20. In the LNP that was the subject of this study, each DE increase of 2 units resulted (on average) in the blockage of an additional 1% of the total lysine. When a CHO system of 100% DE 20 maltodextrin was replaced with a CHO system of 80% DE 5 maltodextrin and 20% sucrose, the LNP lysine blockage was decreased by 831 mg of lysine per 100 g protein, or by 8.25% of total lysine. A blocked lysine reduction of this magnitude (5.68 mmoleslysine per 100 g protein) corresponds to the elimination of 1.75 g of fructoselysine per 100 g protein, and would thereby be expected to impart significant nutritional and tolerance benefits to the low-DE LNP. Furthermore, the correlation between blocked lysine and DE was sufficiently strong to enable a reliable estimation of blocked lysine for a given DE value.

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