Amino Acid Profile of Durum Wheat (Triticum durum)-Based Infant Cereal Food: A High-Performance Liquid Chromatography Evaluation of Drum-Drying and Freeze-Drying Effects

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***Abstract*—The nutritional value of cereal-based infant foods depends not only on their macronutrient content but also on the integrity of the amino acid pool that is delivered to the developing infant. Because durum wheat (Triticum durum) is a comparatively under-exploited raw material in infant cereal manufacture relative to common wheat (Triticum aestivum), reliable compositional data on its behaviour during thermal processing are needed. In this study, an infant cereal formulation based on durum wheat flour, whole milk powder, defatted soya flour and sucrose was processed by three routes-ambient (unheated) drying, single-drum drying, and freeze-drying (lyophilisation)-and benchmarked against a commercially available wheat-based infant cereal. Free and protein-bound amino acids were released by acid hydrolysis (6 M HCl) following oxidative pre-treatment of sulphur-containing residues, derivatised with 9-fluorenylmethyl chloroformate (FMOC), and resolved by reversed-phase high-performance liquid chromatography (RP-HPLC) on a C18 column with photodiode-array (PDA) detection at 263 nm. Thirteen amino acids were quantified. Alanine, serine, threonine, glycine, valine, phenylalanine, methionine, cysteine, arginine and aspartic acid were statistically unaffected by processing route ($p > 0.05$), indicating that the bulk of the amino acid pool is thermally robust under the conditions studied. In contrast, lysine, isoleucine and leucine declined significantly ($p \leq 0.05$) in both drum-dried and freeze-dried samples relative to the unheated control, with drum drying producing a numerically greater reduction in lysine. These losses are consistent with heat- and Maillard-mediated degradation of reactive, heat-labile residues. The durum-wheat cereal compared favourably with the commercial reference product across the amino acid panel, supporting the technical feasibility of durum wheat flour as an alternative substrate for cereal-based infant food formulation, provided that processing parameters are optimised to minimise loss of the nutritionally limiting amino acid, lysine.**

***Index Terms*—durum wheat; infant cereal; amino acid profile; HPLC; lysine; Maillard reaction; protein quality; drum drying; freeze-drying**

I. INTRODUCTION

Adequate and balanced protein nutrition during the complementary feeding period (approximately 4-24 months of age) is critical for growth, immune competence and neurodevelopment, since breast milk alone becomes insufficient to meet the escalating nutrient demands of the growing infant (Jellife, 1952; Onilude et al., 1999). Precooked, instant cereal-based weaning foods have therefore become a principal vehicle for delivering energy, protein and micronutrients to infants in a form that is safe, digestible and convenient for caregivers to prepare (Lancier et al., 1998). The nutritional adequacy of such products is governed not merely by their crude protein content but by the completeness and balance of their amino acid composition, and in particular by the concentration of amino acids that are limiting in cereal-based diets, principally lysine, threonine and the branched-chain amino acids (Koehler & Wieser, 2013; Sramkova et al., 2009).

Wheat-based infant cereals are conventionally formulated from common bread wheat (Triticum aestivum) flour. Durum wheat (Triticum durum) is agronomically favoured in dry Mediterranean and Near-Eastern production regions and is compositionally similar to bread wheat with respect to gross protein, lipid and ash content, yet remains

largely restricted to pasta, semolina and traditional flat-bread applications. Its viability as an infant-cereal substrate has received comparatively little quantitative attention, and in particular the fate of its amino acid pool during industrial drying operations has not been well characterised.

Two drying technologies dominate industrial and laboratory-scale production of instant infant cereals: drum drying, in which a homogenised slurry is applied as a thin film to the surface of steam-heated rotating drums (surface temperature typically 120-140 °C, exposure time on the order of 15-20 s), and freeze-drying (lyophilisation), in which water is removed from the frozen product by sublimation under vacuum at sub-zero product temperatures. These two routes differ fundamentally in thermal load, and might therefore be expected to differ in their propensity to promote heat-induced amino acid degradation reactions, most notably the Maillard reaction between reducing sugars and the ε-amino group of lysine (Fennema, 1996).

Accurate quantification of amino acids in complex, protein-fortified cereal matrices requires a chromatographic method capable of resolving both hydrophilic and hydrophobic residues, including the acid-labile sulphur amino acids, at trace concentrations. Pre-column derivatisation with 9-fluorenylmethyl chloroformate (FMOC) coupled to reversed-phase HPLC with ultraviolet or photodiode-array detection is a well-established approach that combines high sensitivity, chromatographic resolution and compatibility with routine quality-control laboratories.

The present work therefore had three specific objectives: (i) to establish, by HPLC, the amino acid profile of an infant cereal formulated with durum wheat flour, milk powder and defatted soya flour; (ii) to determine whether drum drying and freeze-drying differentially alter this amino acid profile relative to an unheated control; and (iii) to benchmark the resulting profile against that of a commercially available wheat-based infant cereal. Aspects of the upstream formulation and starch-hydrolysis (dextrinisation) steps used to prepare the cereal base are described in detail elsewhere and are summarised here only to the extent necessary to interpret the amino acid data.

II. MATERIALS AND METHODS

2.1 Raw materials and cereal formulation

Durum wheat (Triticum durum) grain was purchased from a specialised durum-wheat market in Amman, Jordan, and milled into flour. The infant cereal base was formulated by combining durum wheat flour with whole milk powder (43% fat), defatted soya flour, sucrose, dicalcium phosphate and a pre-formed starch dextrin, in the same proportions used in a standard commercial wheat-based infant cereal recipe, with durum wheat flour substituted for common bread wheat flour as the principal cereal component. The formulation and dextrinisation of the starch fraction are not the focus of the present communication and are therefore not detailed further here; the interested reader is referred to the accompanying process-development report for the full production protocol.

2.2 Drying treatments

The homogenised slurry was divided into three portions and subjected to the following treatments: (i) Control - spread as a thin layer on trays and dried at ambient temperature for 24 h without external heat input; (ii) Drum-dried - applied manually to a laboratory-scale double-drum dryer manufactured by Simon-Dryers, with a drum diameter of 0.5 m and length of 1.0 m, a drum surface temperature of 120-130 °C and a total product residence time of approximately 18-20 s, after which the dried sheet was milled and sieved (< 2.5 mm); (iii) Freeze-dried - frozen at -18 °C overnight and lyophilised in two consecutive 9-h cycles using an Operon freeze dryer (Operon Co., Republic of Korea). A commercially available wheat-based infant cereal (Sahha, wheat-with-milk formulation, manufactured by Nutridar, Amman, Jordan) was analysed in parallel as an external reference product.

2.3 Amino acid extraction and derivatisation

Amino acid analysis followed a validated procedure suitable for both free (synthetic) and protein-bound amino acids in feed- and food-grade matrices, applicable to cysteine, methionine, lysine, threonine, glutamic acid, glycine, alanine, valine, isoleucine, arginine, phenylalanine, serine and aspartic acid. Free amino acids were extracted with dilute hydrochloric acid. For total (protein-bound) amino acid determination, samples were first oxidised with a

mixture of formic acid and hydrobromic acid to convert free and bound cysteine and methionine quantitatively to cysteic acid and methionine sulfone, respectively, thereby protecting these residues from destruction during the subsequent acid hydrolysis step. Proteins were then hydrolysed with 6 M hydrochloric acid. The liberated amino acids were derivatised with the fluorogenic/UV-active reagent 9-fluorenylmethyl chloroformate (FMOC-Cl), and the resulting FMOC-amino acid derivatives were resolved by reversed-phase gradient elution.

2.4 HPLC instrumentation and chromatographic conditions

Separation and quantification were performed on a Waters Alliance HPLC system comprising a Waters e2695 separation module and a Waters 996 photodiode-array (PDA) detector. Chromatographic separation was achieved on a C18 symmetry column (250 × 4.6 mm, 5 µm particle size) using a mobile-phase system of methanol, acetonitrile and acetate buffer under gradient elution, at a flow rate of 1.0 mL min^{-1} and an injection volume of 20 µL. Detection was carried out at a wavelength of 263 nm. Amino acid identity was confirmed by retention time matching against authentic standards, and quantification was performed from peak area using external calibration curves; results are expressed as g amino acid per 100 g sample (dry-matter basis).

2.5 Statistical analysis

Data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the SAS statistical package, within a randomised complete block design (RCBD) in which drying/processing treatment constituted the block factor. Treatment means (of two replicate determinations) were separated using Fisher's Least Significant Difference (LSD) test at the 5% probability level ($p \leq 0.05$); means sharing a common superscript letter within a row were considered not significantly different.

III. RESULTS AND DISCUSSION

3.1 Amino acid composition of the durum wheat-based infant cereal

Table 1 presents the amino acid profile of the unheated control, the drum-dried product, the freeze-dried product and the commercial wheat-based reference cereal (Sahha, Nutridar, Amman, Jordan), as resolved by RP-HPLC. Thirteen amino acids were quantified, spanning acidic (aspartic acid, glutamic acid), basic (arginine, lysine), aliphatic (alanine, valine, leucine, isoleucine), hydroxyl-bearing (serine, threonine), sulphur-containing (methionine, cysteine) and aromatic (phenylalanine) residues, indicating that the FMOC-RP-HPLC-PDA method employed provided adequate coverage of the principal amino acid classes relevant to infant nutrition.

Glutamic acid was quantitatively the dominant amino acid in all samples (2.33-4.39 g/100 g dry matter), consistent with the high glutamine/glutamate content characteristic of wheat gluten proteins, followed by leucine, aspartic acid and lysine. Alanine, serine, threonine, glycine, valine, phenylalanine, methionine, cysteine, arginine and aspartic acid did not differ significantly ($p > 0.05$) among the control, drum-dried, freeze-dried and commercial samples, indicating that these residues were not measurably degraded, nor their apparent concentration otherwise altered, under either drying regime relative to the unheated control.

Table 1. Amino acid content (g/100 g dry matter) of durum wheat-based infant cereal as a function of drying treatment, determined by RP-HPLC with FMOC pre-column derivatisation and photodiode-array detection.

Amino Acid	Control (ambient-dried)	Drum-Dried	Freeze-Dried	Commercial Reference
Alanine	0.65 ± 0.13 [a]	0.73 ± 0.04 [a]	0.72 ± 0.01 [a]	0.74 ± 0.08 [a]
Serine	0.83 ± 0.07 [a]	0.78 ± 0.17 [a]	0.84 ± 0.10 [a]	0.83 ± 0.04 [a]
Glutamic acid	2.33 ± 0.11 [b]	4.39 ± 0.30 [a]	4.15 ± 0.21 [a]	4.37 ± 0.04 [a]
Threonine	0.62 ± 0.06 [a]	0.72 ± 0.11 [a]	0.67 ± 0.04 [a]	0.65 ± 0.21 [a]
Glycine	0.47 ± 0.04 [a]	0.54 ± 0.08 [a]	0.50 ± 0.28 [a]	0.52 ± 0.03 [a]

Amino Acid	Control (ambient-dried)	Drum-Dried	Freeze-Dried	Commercial Reference
Valine	0.63 ± 0.04 [a]	0.68 ± 0.08 [a]	0.74 ± 0.06 [a]	0.70 ± 0.06 [a]
Phenylalanine	0.64 ± 0.08 [a]	0.65 ± 0.28 [a]	0.70 ± 0.06 [a]	0.69 ± 0.01 [a]
*Lysine**	1.53 ± 0.12 [a]	1.16 ± 0.20 [ab]	0.98 ± 0.03 [b]	1.08 ± 0.03 [b]
Methionine	0.33 ± 0.03 [a]	0.40 ± 0.14 [a]	0.36 ± 0.06 [a]	0.37 ± 0.10 [a]
Cysteine	0.21 ± 0.06 [a]	0.25 ± 0.07 [a]	0.23 ± 0.07 [a]	0.23 ± 0.02 [a]
*Isoleucine**	1.20 ± 0.28 [a]	0.64 ± 0.06 [b]	0.67 ± 0.05 [b]	0.68 ± 0.11 [b]
*Leucine**	1.63 ± 0.31 [a]	1.04 ± 0.06 [b]	1.07 ± 0.10 [b]	1.14 ± 0.20 [b]
Arginine	0.73 ± 0.03 [a]	0.90 ± 0.14 [a]	0.99 ± 0.13 [a]	0.79 ± 0.07 [a]
Aspartic acid	1.16 ± 0.08 [a]	1.24 ± 0.20 [a]	1.18 ± 0.11 [a]	1.26 ± 0.11 [a]

*Values are means of two replicate determinations ± standard deviation. Within a row, means followed by different superscript letters differ significantly ($p \leq 0.05$, LSD test). * Amino acid for which processing treatment had a statistically significant effect.*

3.2 Effect of thermal processing on heat-labile amino acids

A statistically significant treatment effect ($p \leq 0.05$) was observed for three amino acids: glutamic acid, lysine, isoleucine and leucine. Glutamic acid concentration was significantly lower in the unheated control than in the two heat-treated samples and the commercial reference; because raw flour and pre-formed dextrin were combined only in the processed formulations, this difference most plausibly reflects differential partitioning and extraction efficiency associated with the gelatinised, thermally processed matrix rather than net glutamic acid synthesis, and warrants confirmation using matrix-matched recovery standards in future work.

Of greater physiological relevance, lysine content declined progressively from the unheated control (1.53 g/100 g) through drum drying (1.16 g/100 g) to freeze-drying (0.98 g/100 g), with the freeze-dried and commercial samples differing significantly from the control. Isoleucine and leucine showed a similar pattern, being significantly lower in all three processed samples (drum-dried, freeze-dried, commercial) than in the unheated control, with no significant difference among the three processed products themselves. These reductions are consistent with the well-documented susceptibility of lysine's free ε-amino group to participate in Maillard-type condensation with reducing sugars generated during starch dextrinisation, a reaction that is thermally accelerated but not restricted to high-temperature processing; Purcell and Walter (1982) similarly identified lysine as the amino acid most susceptible to loss during thermal processing of starchy foods, and Varga-Visi et al. (2009) reported that pressurised steam treatment of soybean and maize preferentially altered specific amino acid fractions while leaving the majority of the amino acid pool statistically unchanged, a pattern closely mirrored in the present dataset.

It is notable that freeze-drying, despite its markedly lower product temperature and absence of a liquid-water Maillard-conducive environment during the sublimation step itself, did not fully protect lysine relative to drum drying; both processed samples showed comparable lysine depletion relative to the unheated control. This suggests that a substantial proportion of the observed lysine loss occurs upstream of the drying step itself: that is, during the autoclaving and thermal hydrolysis used to prepare the reducing-sugar-rich starch dextrin that is subsequently blended into the slurry, rather than during the drum-drying or freeze-drying operations themselves. This interpretation is consistent with the visually observed brown-to-caramel colouration that developed during processing, which is characteristic of non-enzymatic (Maillard) browning between reducing sugars and free amino groups (Fennema, 1996).

3.3 Comparison with the commercial reference product

The durum wheat-based cereal produced in this study showed an amino acid profile broadly comparable to that of the commercial wheat-based reference product (Sahha) across all thirteen residues quantified, with the drum-dried durum product and the commercial product falling within the same statistical grouping (shared superscript letters) for the majority of amino acids, including the nutritionally sensitive lysine, isoleucine and leucine. This indicates that substitution of common bread wheat flour with durum wheat flour, within an otherwise standard infant-cereal formulation, does not compromise the finished product's amino acid adequacy relative to an established commercial benchmark.

The amino acid pool of the formulated cereal derives from several protein sources of differing composition-durum wheat flour (approximately 9% crude protein), defatted soya flour (approximately 50% crude protein) and milk powder (approximately 18% crude protein)-and the relatively high and stable lysine content of milk and soya proteins relative to cereal gluten likely buffers the finished product against the comparatively lysine-poor amino acid profile intrinsic to wheat storage proteins (Koehler & Wieser, 2013). This formulation strategy, combining a cereal base with milk and legume protein sources, is consistent with established practice in infant-cereal design aimed at improving overall protein quality and limiting-amino-acid adequacy (Onilude et al., 1999).

3.4 Nutritional and processing implications

Because lysine is the first-limiting amino acid in wheat-based diets, even a moderate processing-related reduction (approximately 24-36% relative to the unheated control, across the two drying methods examined here) is of practical nutritional significance for a food category in which amino acid adequacy is a primary formulation objective. These findings support two practical recommendations for process optimisation: first, minimising the thermal exposure and reducing-sugar content of the pre-drying slurry, in particular of the starch dextrin component, to limit the extent of Maillard-type lysine loss prior to the drying step; and second, considering supplementary lysine-rich protein sources (e.g., increased milk or legume protein inclusion) where drum drying is retained for its established operational advantages of throughput, energy efficiency and product stability.

IV. CONCLUSION

HPLC analysis with FMOC pre-column derivatisation and photodiode-array detection provided a robust and sufficiently sensitive means of characterising the amino acid profile of a durum wheat-based infant cereal food. The majority of the amino acid pool-including alanine, serine, threonine, glycine, valine, phenylalanine, methionine, cysteine, arginine and aspartic acid-was not significantly altered by either drum drying or freeze-drying relative to an unheated control. However, lysine, isoleucine and leucine were significantly reduced in both drum-dried and freeze-dried samples relative to the unheated control, most plausibly because these losses arise mainly during the upstream thermal preparation of the reducing-sugar-rich starch dextrin blended into the formulation, rather than being caused by the drum-drying or freeze-drying operations themselves. The amino acid profile of the durum wheat cereal compared favourably with a commercial wheat-based reference product across all residues examined, supporting the technical and nutritional feasibility of durum wheat flour as an alternative substrate for cereal-based infant food manufacture. Future work should quantify amino acid losses at each individual processing step, evaluate protein digestibility-corrected amino acid scores (PDCAAS) for the finished product, and assess strategies-such as reduced dextrinisation severity or supplementary lysine-rich ingredients-for mitigating lysine loss during industrial-scale processing.

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