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Non-protein nitrogen in infant cereals affected by industrial processing

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Abstract

The effects of industrial processing on non-protein content in four varieties of infant cereals, so-called 'Multicereal' and 'Wheat' as gluten infant cereals, and 'Growth' and 'Rice and carrot' as gluten-free infant cereals were determined. Samples were classified according to their industrial stage of treatment: (1) mixture of raw flours; (2) mixture of roasted flours; (3) mixture of enzymatically hydrolysed and drum-dried flours (film); (4) commercial infant cereals. Total nitrogen (TN), protein nitrogen (PN) and non-protein nitrogen (NPN) contents were higher in gluten infant cereals than in gluten-free infant cereals. NPN content was always higher than PN content in all cereals. Industrial processing led to a significant increase in NPN ($P < 0.01$) and free amino acid contents ($P < 0.001$), whereas TN and PN contents decreased significantly ($P < 0.05$). Although non-amino acid nitrogen components remained stable with the industrial processing, a high content of ammonium was found in gluten-free infant cereals as a consequence of taurine and asparagine degradation.

Keywords: Infant cereals; Total nitrogen; Protein nitrogen; Non-protein nitrogen; Free amino acids; Non-amino acids nitrogen components

1. Introduction

Besides protein nitrogen (PN), non-protein nitrogen (NPN) represents an important fraction in vegetables that determines protein quality (Bhatty, 1973; Periago, Ros, Martinez, & Rincón, 1996b). NPN involves a wide group of components, including urea, ammonium salts, single amino acids, small peptides, amines, amides and nucleotides (Goedhart & Bindels, 1994), and all them end in ammonium as the final chemical form. Several NPN components with important functions in infant nutrition have been identified in human mature milk (Atkinson, Schnurr, Donovan, & Lönnerdal, 1989). Among these, taurine and several other nucleotides are probably involved in the development of the newborn infant. In addition, polyamines, such as spermine, spermidine or putresceine, have clear trophic characteristics toward cell proliferation and differentiation in many tissues, including the gastrointestinal tract (Pollack, Koldovsky, & Nishioka, 1992). For these reasons, NPN has been claimed to have conditionally essential substances for infant feeding (Romain, Dandrifosse, Jeusette, & Forget, 1992). Since during the past decade the consumption of manufactured infant foods in industrialised countries has been increased, it is important to be more concerned with the assessment of the nutritional values of such foods, especially in the interpretation of food tables. Infant cereals are the commonest food recommended by paediatricians to start the weaning feeding at the age of 4–6 months due to their high energetic load, based on their carbohydrate and protein contents (77–78% and 12–13%, respectively) (Molina & Maldonado, 1993). Infant cereal protein quality depends on cereal selection and ingredient quality and 'technological treatments' (Pompei, Rossi, & Mare, 1987; Pérez-Conesa, Ros, & Periago, 2002). Some of the cereals used to formulate infant cereals, such as wheat, corn or oat, have high levels of the amide-containing amino acids (glutamine and

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asparagine), that are susceptible to hydrolysis by thermal or enzymatic deamidation, which leads also to the release of ammonium (Riha, Izzo, Zhang, & Ho, 1996). In addition, industrial processing such as severe heating (>70 °C) long period of time (> 45 min), is known to induce chemical changes in proteins and, among other changes, produces amino acid racemisation, a process which may impair the nutritional value and safety of foods (Man & Bada, 1987). These interactions take place between the lateral groups of the amino acids, that lead to the formation of the so called 'unnatural' amino acids, such as lanthionine, β-aminoalanine, ornithine, ornithinoalanine, diaminopropionic acid and particularly lysinoalanine, which is known to decrease the nutritional value of foods (Hurrell & Finot, 1983; Klostermeyer & Reimerdes, 1977). On the other hand, urea nitrogen contributes to the pool available for synthesis of non-essential amino acids (Fommon et al., 1988). In previous studies we have analyzed the nutritional value of the carbohydrates (Bernal, Periago, & Ros, 2002) and protein (Periago, Ros, Martinez, & Rincón, 1992; Periago et al., 1996a; Ros & Rincón, 1993) in infant cereals and legumes during industrial processing. More nutritional information could be obtained by the evaluation of the NPN. The objective of this research was to evaluate the NPN changes during the main industrial processing steps of commercial infant cereals.

2. Materials and methods

2.1. Samples and processing conditions

Two commercial infant cereals with gluten (gluten infant cereals), ('Multicereal and 'Wheat'), and two gluten-free (gluten-free infant cereals) ('Growth' and 'Rice with carrot') were provided by Hero España Co. (Murcia, Spain) and they were evaluated at four stages of their industrial processing (raw flour, roasted flour, enzymatically hydrolysed and drum-dried and commercial infant cereals). Table 1 shows the different ingredients used in the infant cereal composition, and Fig. 1 the flow diagram followed to obtain the infant cereals. A mix of raw flours was roasted at 120 °C for 30 min. Subsequently, the product was enzymatically treated in water with α-amylase from *Bacillus* sp. (Sigma, St. Louis USA), to hydrolyse the starch chains to dextrins, with the aim to make the cereal more digestible in the infant's gut, and drum-dried to inactivate the enzyme and to obtain a thin film of cereal. Sugar and minerals and vitamins were also incorporated as pow-

Table 1
Infant cereals composition

Ingredients	Infant cereal			
	Gluten		Gluten-free	
	'Multicereal'	'Wheat'	'Growth'	'Rice and carrot'
Cereals				
• Wheat	+	+	–	–
• Millet	+	–	–	–
• Barley	+	–	–	–
• Rye	+	–	–	–
• Sorghum	+	–	–	–
• Rice	+	–	+	+
• Oats	+	–	–	–
• Maize	+	–	+	+
Other ingredients				
• Carrot	–	–	–	+
• Tapioca starch	–	–	+	–
• Maize starch	–	–	+	–
• Malt extract	–	+	–	–
• Vanilla	+	+	+	–
• Saccharose	+	+	+	+
Minerals and vitamins				
• Reduced iron	+	+	+	+
• Calcium phosphate	+	+	+	+
• Potassium iodide	–	–	+	–
• Vitamin supplement[a]	+	+	+	+

[+]Ingredient included in the formulation.
[–]Ingredient not included in the formulation.
[a] Vitamin supplement: vitamin A 1000 IU; vitamin D 300 IU; vitamin E 10 mg; vitamin C 90 mg; vitamin B_1 1 mg; vitamin B_2 1.2 mg; vitamin B_6 1 mg; vitamin B_{12} 1.2 μg; niacin 12 mg; pantothenic acid 6 mg; folic acid 30 μg and biotin 40 μg.

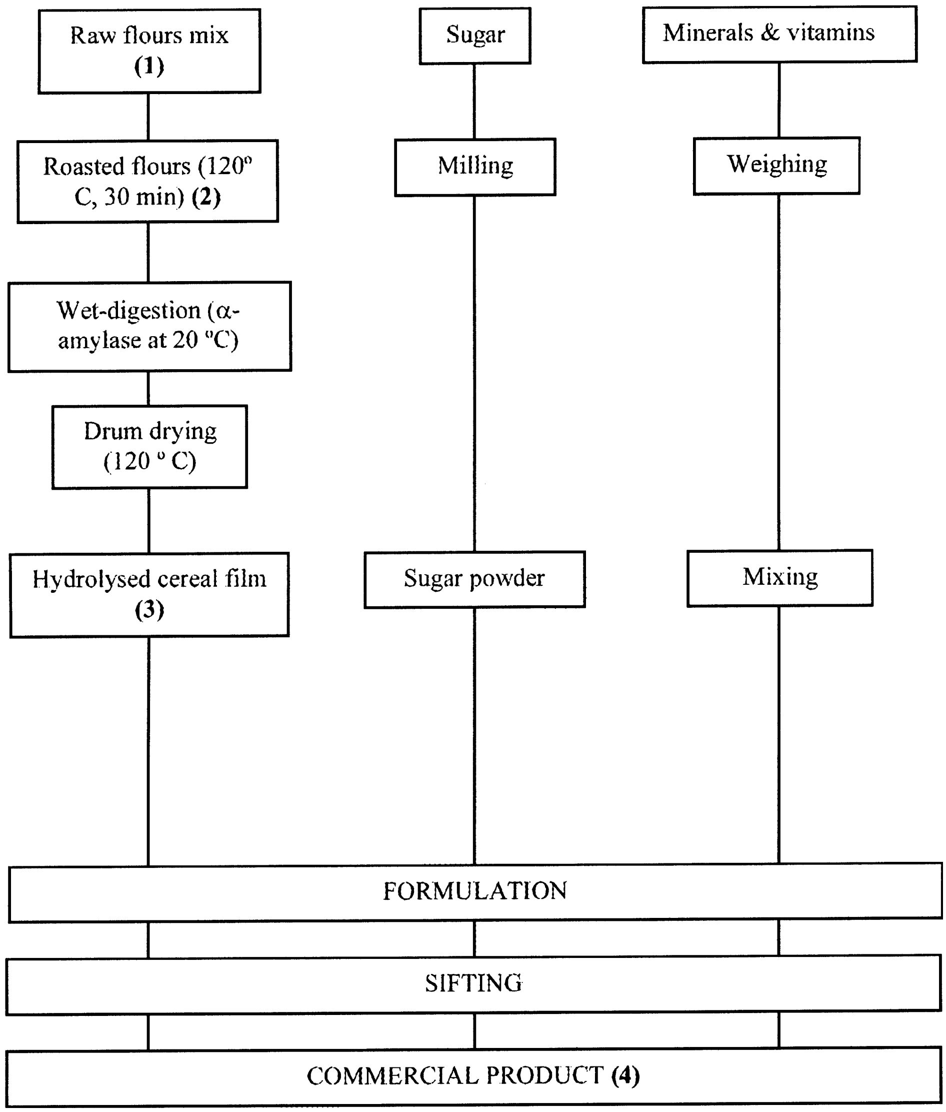

Fig. 1. Flow diagram in the manufacture of infant cereals, showing the four stages of processing as numbers 1–4 (1, mixture of raw flours; 2, mixture of roasted flours; 3, mixture of enzymatically hydrolysed and drum-dried flours; 4, commercial infant cereals).

der. After the formulation of the different ingredients, all of them were sifted using an air-powdered mixer and the final mixture obtained was also called commercial infant cereal.

All the samples were stored at room temperature in tightly closed plastic containers with desiccant to avoid moisture damage prior to analyses. Analyses were done in triplicate for each method and results are expressed on a dry-weight basis.

2.2. Chemical analysis

Total nitrogen (TN) (nitrogen from crude protein) was determined according to the micro-Kjeldahl method (Association of Official Analytical Chemists, AOAC, 1990). PN (nitrogen from true protein) content was determined by the trichloroacetic acid (TCA) precipitation method (Awolumate, 1983). 100 mg of sample was solubilized in 5 ml of a 0.2% NaOH (w/v) solution, shaken for 20 min, and centrifuged at 6000*g* for 5 min at room temperature. The pellet was washed with 4 ml of 0.2% NaOH solution and added to the supernatant after a second centrifugation under the same conditions. TCA (6 ml) was added to the collected supernatants and left for 2 h at 4 °C occasional shaking prior to centrifugation at 12,000*g* for 20 min at room temperature. The precipitate was dried for 1 h at 105 °C and weighed. Nitrogen content of the precipitate was then determined by the micro-Kjeldahl method. NPN was calculated as the difference between TN (micro-Kjeldahl method) and PN (Awolumate method) as recommended by Periago et al. (1996b). Free amino acids (FAA) were determined using the ninhydrin method (Luse, 1976). 10 mg of sample were extracted with 100 ml of destilled-deionized water and centrifuged at 6000*g* for 5 min at room temperature. Aliquots of 1 ml of supernatant were mixed with 1 ml of ninhydrin reagent (Sigma Chemical Co., St. Louis, MO). The solution was boiled for 20 min, cooled at room temperature, and 5 ml of 500 g/l iso-propanol were mixed with the solution. The absorbance was measured at 570 nm on a Hitachi spectrophotometer model U-2000 (Tokyo, Japan). An equimolar mixture of amino acids was used as standard (Sigma Chemical Co., St. Louis, MQ).

2.3. Non-amino acid nitrogen components (NAANC), ammonium and asparagine contents

In order to prepare the sample, cereal flours were hydrolyzed with 6 N HCl at 110 °C for 24 h in amber vacuum sealed tubes, following the method of Moore and Stein (1963). After hydrolysis, samples were filtered through 0.22 μm Millipore filters, diluted to 2 ml and pH adjusted to 2.2 with 0.2 M lithium citrate loading buffer (pH 2.2) (Pharmacia LKB Biochrom Ltd., Cambridge, England) prior to loading into the ion-exchange column. NAANC, ammonium and asparagine contents were determined with an amino acid analyser LKB Alpha Plus (Pharmacia LKB Biochrom Ltd., Cambridge, England), comparing the chromatogram of the samples with a standard solution (Pharmacia LKB Biochrom Ltd., Cambridge, England).

2.4. Statistical analysis

Results were expressed as the mean values and standard deviations of three determinations. The statistical analyses were carried out using the one-way ANOVA test at a significance level of $P < 0.05$. To ascertain the significance among means of the samples, the Tukey's means separation test, with a significant level of $P < 0.05$, was applied. To explain the relationships between modifications on the different parameters studied, a correlation analysis (Pearson's correlation coefficient) was carried out. Statistical analyses of the data were performed using a SPSS programme version 10.0 for windows (SPSS Inc.Chicago, I.C.).

3. Results and discussion

3.1. Effect of industrial processing on TN, PN, NPN and FAA contents of infant cereals

Table 2 presents the TN, PN, NPN and FAA contents of the infant cereals studied according to industrial processing. Both heat and enzymatic treatments significantly modified ($P < 0.05$) the parameters studied in all infant cereals. 'Multicereal' and 'Wheat' (gluten infant cereals) showed higher TN, PN and NPN contents than 'Growth' and 'Rice and carrot' (gluten-free infant cereals). Gluten infant cereals showed values above 1.55%, 0.21% and 0.87%, respectively, while gluten-free infant cereals showed values above 1.11%, 0.08% and 0.84%, respectively. An important finding is that NPN content was always higher than PN content. This effect might be attributed to the hydrolysis of cereal protein, since these treatments release peptides and FAA from peptidic chains (Dahlin & Lorenz, 1993), an effect that has also been noted in legumes, such as peas (Periago et al., 1998). Both TN and PN contents decreased with the industrial treatment, showing a greater effect on the PN in gluten-free infant cereals, ranging from 0.32 to 0.08 g/100 g in 'Growth' and from 0.48 to 0.09 g/100 g in 'Rice and carrot'. To achieve a closer view of the industrial processing, the correlations between PN and NPN, and PN and FAA (Table 3) have been studied. As a general rule content of PN was significantly but negatively correlated with NPN content in all infant cereals as well as with FAA with the exception of 'Multicereal' infant cereals. If we plot the NPN/PN ratio during the industrial processing (Fig. 2), it can be observed that it is actually not until stage 3 (enzymatically hydrolysed and drum-dried) that the ratio does not show a different trend. Following the lines of the graph, the NPN/PN ratio showed a similar tendency to increase, 'Growth' being the highest and 'Multicereal' the lowest. At commercial product (stage 4), all infant cereals maintained the trend to increase, especially notable in gluten-free infant cereals. As shown in Table 2, as NPN content increased, FAA content also increased during the industrial treatment, due to the releasing effects mainly, of enzymatically hydrolysed and drum dried (stage 3) processing, where it is at least double that in the previous stage (roasting or stage 2). This effect on FAA is important but still there is a large proportion of NPN, not FAA, that may have an important impact on nutritional value of the infant cereals, and that justifies the evaluation and discussion in the next section of the paper.

If we consider that all TN is protein, and multiply TN by 5.7, raw cereals itself (stage 1) will provide a reasonable amount of protein (from 7.18% in the lowest, in 'Growth' to 11.3%, the highest in 'Wheat') as well as commercial infant cereal (stage 4) (6.32% in 'Growth' to 10.3% in 'Wheat'). However, if we split PN from TN, true protein (as PN $\times$ 5.7) will be reduced significantly. As an example, in commercial infant cereals the true protein content will be 1.31% in 'Multicereal, 1.19% in 'Wheat', 0.45% in 'Growth' and 0.51% in 'Rice and carrot'. This drastic reduction leads an important content of NPN. This finding underlines the significance of NPN as an indicator of protein quality especially in food with sensitive population as a

Table 2
TN, PN, NPN and FAA contents of infant cereals according to the industrial processing

Infant cereal	Nitrogen quality parameters							
	TN(g/100 g)	S^{B}	PN (g/100 g)	S	NPN(g/100 g)	S	FAA(g/100 g)	S
'*Multicereal*'								
1[A]	1.55 ± 0.04^{b}		0.68 ± 0.28^{a}		0.87 ± 0.24^{b}		0.100 ± 0.024^{b}	
2	1.63 ± 0.16^{ab}	*	0.47 ± 0.14^{ab}	*	1.16 ± 0.01^{ab}	**	0.082 ± 0.027^{b}	***
3	1.77 ± 0.01^{a}		0.34 ± 0.07^{ab}		1.43 ± 0.076^{a}		0.170 ± 0.003^{a}	
4	1.59 ± 0.04^{ab}		0.23 ± 0.03^{b}		1.36 ± 0.01^{a}		0.214 ± 0.003^{a}	
'*Wheat*'								
1	1.98 ± 0.04^{b}		0.62 ± 0.16^{a}		1.36 ± 0.12^{b}		0.068 ± 0.007^{c}	
2	2.09 ± 0.01^{a}	***	0.57 ± 0.15^{a}	**	1.52 ± 0.14^{ab}	**	0.056 ± 0.003^{c}	***
3	2.01 ± 0.05^{ab}		0.25 ± 0.03^{b}		1.76 ± 0.02^{a}		0.176 ± 0.004^{b}	
4	1.81 ± 0.02^{c}		0.21 ± 0.02^{b}		1.60 ± 0.01^{ab}		0.196 ± 0.004^{a}	
'*Growth*'								
1	1.26 ± 0.06^{a}		0.32 ± 0.07^{a}		0.94 ± 0.01^{d}		0.076 ± 0.002^{b}	
2	1.29 ± 0.06^{a}	**	0.29 ± 0.06^{a}	**	0.99 ± 0.01^{c}	***	0.070 ± 0.001^{b}	***
3	1.18 ± 0.02^{ab}		0.12 ± 0.01^{b}		1.06 ± 0.01^{a}		0.143 ± 0.007^{a}	
4	1.11 ± 0.04^{b}		0.08 ± 0.04^{b}		1.03 ± 0.01^{b}		0.140 ± 0.002^{a}	
'*Rice and carrot*'								
1	1.32 ± 0.01^{b}		0.48 ± 0.10^{a}		0.84 ± 0.09^{b}		0.36 ± 0.002^{c}	
2	1.41 ± 0.01^{a}	***	0.31 ± 0.09^{ab}	**	1.10 ± 0.08^{a}	***	0.038 ± 0.002^{c}	***
3	1.37 ± 0.04^{ab}		0.15 ± 0.03^{bc}		1.21 ± 0.01^{a}		0.107 ± 0.013^{a}	
4	1.24 ± 0.02^{c}		0.09 ± 0.01^{c}		1.15 ± 0.01^{a}		0.301 ± 0.022^{a}	

[a–d] Means (±SD) of three determinations on dry weight basis. Different letters within the same column indicate significant differences at $P < 0.05$ for each type of infant cereals.

[A] Industrial processing: (1) mixture of raw flours; (2) mixture of roasted flours; (3) mixture of enzymatically hydrolysed and drum-dried flours; (4) commercial infant cereals.

[B] S, Statistical differences by analysis of variance; NS, non-significant for $P > 0.05$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P > 0.001$.

Table 3
Correlation factor r and degree of significance of PN versus NPN, and PN versus FAA contents in relation to the industrial processing of the four infant cereals

Infant cereal	Parameters			
	PN–NPN		PN–FAA	
	r	Significance	r	Significance
'Multicereal'	−0.893	0.000	−0.478	NS
'Wheat'	−0.891	0.000	−0.867	0.000
'Growth'	−0.801	0.002	−0.893	0.000
'Rice and carrot'	−0.913	0.000	−0.730	0.007

NS, non-significant for $P > 0.05$.

target, such as babies or infants, and demands a cautious interpretation of food composition tables. No reference has been found to support our results for infant cereals in the literature but similar data can be obtained in legume studies (Periago et al., 1992).

3.2. *Effect of industrial processing on non-amino acid nitrogen components*

Many NAANC from human milk, which constitute about 25% of the TN, have been identified (Harzer, Franzke, & Bindels, 1984). It is difficult to exhaustively list all the NAANC, but the most important would include low molecular weight peptide hormones, taurine, carnithine, creatine and creatinine, polyamines, the amino sugars *N*-acetylglucosamine and *N*-acetylneuraminic acid (sialic acid), amino alcohols, choline and ethanolamine, nucleic acids and nucleotides, free amino acids, urea, uric acid and ammonium (Atkinson et al., 1989). It is known that some of these components (taurine, carnithine, polyamines, choline and nucleotides) are 'conditionally essential' for infant nutrition (Gil Hernández & Gil Campos, 2001). However, there is very little information about these components in cereals and the way that industrial processing affects NAANC of infant cereals. Since FAA has been described in the previous section, only the remaining NAANC will be presented. Table 4 presents the content of the main NAANC available for study (Ans, anserine; Car, carnosine; Cis, cistationine; Cit, citruline acid; Pet, phosphoethanolamine; Pse, phosphoserine; Sar, sarcosine; Tau, taurine) in infant cereals during industrial processing. Some NAANC such as hydroxyproline, α-aminoadipic acid, α-aminobutyric acid, γ-aminobutyric acid, hydroxylysine, ornithine (orn) 1-methyl-histidine, 3-methyl-histidine were also determined, but due to their very low amounts in our samples (less than 0.1 g/100 g protein) and their little importance in infant nutrition, their values are not shown.

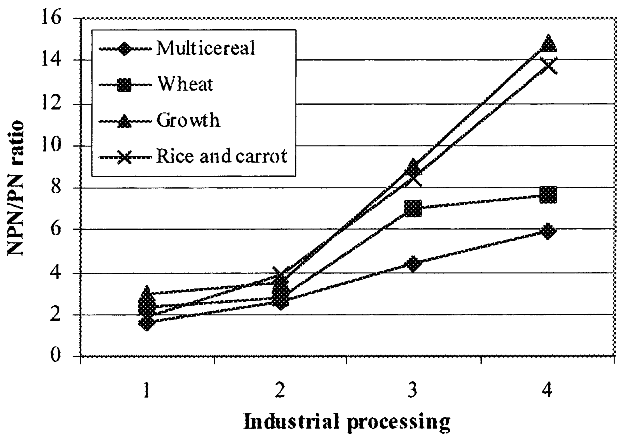

Fig. 2. NPN and PN ratio of the infant cereals in the four stages of industrial processing of the infant cereals (mixture of raw flours (1), mixture of roasted flours (2), mixture of enzymatically hydrolysed and drum–dried flours (3), and commercial infant cereals (4)).

In general the NAANC content in infant cereals did not show significant variations with the industrial processing. The only components affected (significantly increasing their contents), were Cis in 'Wheat' ($P < 0.01$) and Sar in 'Multicereal' ($P < 0.05$) (Table 4). The most abundant NAANC was Pet (23.1 and 22.8 g/100 g protein in raw flours of 'Growth' and 'Rice and carrot', respectively), which comes from ethanolamine. In addition, another compound derived from ethanolamine is choline, which is considered essential for infant development (Zeisel, 2000). After Pet, Tau is the most representative NAANC (6.77 g/100 g protein in commercial 'Multicereal' and 6.71 g/100 g protein in film of 'Wheat') although it is only one-fourth of the Pet content. Tau is very important in cerebellar and vision development and, together with Tyr and Cys, is considered 'conditionally essential' in preterm newborns (Mahan & Escott-Stump, 1998). Tau content in human milk is 0.5 g/ l00 g protein, and it is usually added to infant formula at 0.27 g/ l00 g protein. The results also show that, while in gluten infant cereals the amount of Tau is important as described, in gluten-free infant cereals there was an absence of this NAANC (Tables 4 and 5). Therefore, it is possible that, in gluten-free infant cereals, Tau was transformed into ammonium with heat treatment. Cit is the third part of NAANC, mainly at 2.89 g/ l00 g protein in film of 'Growth' and 3.24 g/100 g protein in raw flours of 'Rice and carrot'. The nutritional interest in Cit is that the compound is catabolized in the liver

Table 4
Non-amino acid nitrogen components content (g/100 g of protein) at the four stages the of industrial processing of 'Multicereal', 'Wheat', 'Growth' and 'Rice and carrot' infant cereals

Infant cereal	Ans	Car	Cit	Cis	Pet	Pse	Sar	Tau
'*Multi cereal*'								
1[A]	0.78 ± 0.26^a	0.53 ± 0.91^a	0.44 ± 0.29^a	0.36 ± 0.05^a	0.58 ± 0.40^a	0.51 ± 0.20^a	0.73 ± 0.38^c	5.84 ± 2.21^a
2	1.53 ± 0.29^a	0.76 ± 0.23^a	0.24 ± 0.09^a	0.26 ± 0.12^a	0.58 ± 0.29^a	0.53 ± 0.26^a	0.85 ± 0.07^{ab}	4.98 ± 1.68^a
3	0.28 ± 0.48^a	1.58 ± 0.05^a	0.09 ± 0.17^a	0.23 ± 0.40^a	0.20 ± 0.11^a	0.36 ± 0.16^a	1.08 ± 0.55^{ab}	5.59 ± 1.13^a
4	1.98 ± 1.66^a	1.55 ± 0.94^a	0.26 ± 0.10^a	0.13 ± 0.22^a	0.93 ± 0.53^a	0.59 ± 0.18^a	1.84 ± 0.40^a	6.77 ± 1.35^a
'*Wheat*'								
1	1.30 ± 0.64^a	0.94 ± 0.54^a	0.10 ± 0.18^a	0.21 ± 0.08^b	0.55 ± 0.28^a	0.67 ± 0.09^a	0.52 ± 0.16^a	5.72 ± 1.22^a
2	1.38 ± 0.68^a	1.13 ± 0.34^a	0.09 ± 0.17^a	0.33 ± 0.16^b	BDL	0.67 ± 0.11^a	1.54 ± 1.10^a	4.92 ± 2.51^a
3	1.09 ± 0.74^a	0.64 ± 0.34^a	0.08 ± 0.03^a	0.35 ± 0.01^b	0.18 ± 0.31^a	0.44 ± 0.01^a	2.03 ± 1.17^a	6.71 ± 0.59^a
4	0.42 ± 0.33^a	0.98 ± 0.56^a	0.04 ± 0.07^a	0.71 ± 0.14^a	0.30 ± 0.00^a	0.39 ± 0.23^a	1.29 ± 0.89^a	6.21 ± 0.94^a
'*Growth*'								
1	0.23 ± 0.40^a	0.21 ± 0.37^a	BDL[B]	0.45 ± 0.04^a	23.12 ± 5.51^a	3.38 ± 1.94^a	0.61 ± 1.05^a	BDL
2	1.05 ± 0.36^a	0.12 ± 0.21^a	1.65 ± 2.86^a	0.44 ± 0.05^a	10.34 ± 3.15^a	1.23 ± 0.64^a	0.88 ± 0.48^a	BDL
3	0.21 ± 0.37^a	0.59 ± 0.21^a	2.89 ± 0.73^a	0.52 ± 0.13^a	13.21 ± 7.10^a	1.49 ± 0.37^a	0.77 ± 0.20^a	BDL
4	BDL	BDL	BDL	0.32 ± 0.55^a	15.50 ± 7.82^a	1.42 ± 0.13^a	0.46 ± 0.80^a	BDL
'*Rice and carrot*'								
1	0.43 ± 0.43^a	0.14 ± 0.24^a	3.24 ± 0.43^a	1.03 ± 0.46^a	22.84 ± 7.74^a	0.91 ± 0.49^a	0.81 ± 0.15^a	0.24 ± 0.41^a
2	0.23 ± 0.40^a	0.29 ± 0.02^a	BDL	0.89 ± 0.35^a	14.64 ± 1.86^a	0.87 ± 0.99^a	BDL	BDL
3	0.56 ± 0.06^a	BDL	1.10 ± 1.91^a	0.67 ± 0.31^a	10.95 ± 4.33^a	1.86 ± 0.32^a	0.25 ± 0.44^a	BDL
4	0.19 ± 0.33^a	0.09 ± 0.17^a	BDL	0.12 ± 0.21^a	12.02 ± 2.94^a	1.05 ± 0.55^a	0.27 ± 0.46^a	BDL

[a–c]Means ($\pm$SD) of three determinations expressed as g/100 g of protein. Different letters within the same column indicate significant differences at $P < 0.05$.

[A] Industrial processing: (1) mixture of raw flours, (2) mixture of roasted flours, (3) mixture of enzymatically hydrolysed and drum-dried flours, and (4) commercial infant cereals.

[B] Below detection limit. (Ans, Anserine; Car, Carnosine; Cis, Cystathionine; Cit, Citruline acid; Pet, Phosphoethanolamine; Pse, Phosphoserine; Sar, Sarcosine; Tau, Taurine).

Table 5
Ammonium and asparagine contents (g/100 g of protein) at the four stages of the industrial processing of 'Multicereal', 'Wheat', 'Growth' and 'Rice and carrot' infant cereals

Infant cereal	Ammonium	S^B	Asparagine (Asn)	S
'Multicereal'				
1[A]	5.73 ± 1.42^a		6.59 ± 0.25^c	
2	6.23 ± 1.12^a	NS	21.47 ± 2.24^b	***
3	7.17 ± 0.36^a		27.56 ± 0.68^a	
4	6.18 ± 0.71^a		23.09 ± 1.07^b	
'Wheat'				
1	6.91 ± 1.25^a		22.85 ± 0.17^b	
2	7.71 ± 0.69^a	NS	25.82 ± 2.79^{ab}	**
3	8.36 ± 0.38^a		31.38 ± 0.93^a	
4	6.99 ± 1.00^a		27.09 ± 3.11^{ab}	
'Growth'				
1	15.18 ± 1.93^a		0.32 ± 0.16^a	
2	15.16 ± 1.55^a	NS	BDL[C]b	*
3	13.87 ± 1.28^a		BDL[b]	
4	$15.42 \pm 2,25^a$		0.18 ± 0.06^{ab}	
'Rice and carrot'				
1	15.33 ± 0.57^a		8.89 ± 4.44^a	
2	15.28 ± 1.44^a	NS	BDL[b]	*
3	12.81 ± 1.40^a		BDL[b]	
4	14.78 ± 0.61^a		BDL[b]	

[a–c]Means (±SD) of three determinations expressed as g/100 g of protein. Different letters with in the same column indicate significant differences at $P < 0.05$ for each type-of infant cereal.

[A] Industrial processing: (1) mixture of raw flours; (2) mixture of roasted flours; (3) mixture of enzymatically hydrolysed and drum-dried flours; (4) commercial infant cereals.

[B] Statistical differences by analysis of variance: NS, non-significant for $P > 0.05$, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$.

[C] Below detection limit.

and used to form glucose, ketones and fatty acids; meanwhile the majority of the nitrogen is converted into ammonium and is eliminated as urea (Linder, 1988). The remaining NAANC (Pse, Sar, Ans and Car) will provide less than 2 g/100 g of protein. Also a small amount of Orn was detected in 'Rice and carrot' with a mean content of 0.2 g/100 g protein. This component, together with Met is a precursor of polyamines, which have some effects on intestinal and gut microflora development in newborns (Gil, Suárez, & Ramirez-Tortosa, 1999).

3.3. Technological treatment effects on ammonium and asparagine of infant cereals

Ammonium, as a final metabolite of the protein and NPN degradation, can be useful as an indirect indicator of nitrogenous compound quality, and also of interest in the industrial processing of infant cereals. In addition, in vegetables, apart from the breakdown of Asn during maturity (Hahlbrock & Grisebach, 1979; Srivastava & Singh, 1987), ammonium could be generated due to some deamidation reactions of the amide groups of Glu and Asn as a consequence of heat treatment (>100 °C) (Cheftel, Cuq, & Lorient, 1989). For this reason, the contents of ammonium and Asn during industrial processing of infant cereals are represented in Table 5. The results show that ammonium was not affected significantly by industrial processing, while Ans was increased significantly in 'Multicereal' ($P < 0.001$) and 'Wheat' ($P < 0.01$). It is important to note that ammonium content in gluten-free infant cereals was higher than in gluten infant cereals. These values are similar to those found in artichokes after heating (6.78 g/100 g protein) by López et al. (1996). However, their content of Asn was smaller in gluten-free infant cereals than in gluten infant cereals, with values below the detection limit at different stages of growth in ('Rice and carrot'). Asn is a typical amino acid of vegetables such as asparagus, and it may be odd to find it infant cereals. However, it is considered that in wheat proteins a large portion of Asp is under the form of Ans (Cheftel et al., 1989). So, the cereals which have wheat as an ingredient in their composition ('Multicereal' and 'Wheat') showed higher amounts of Ans (Table 4), and a smaller Asp content (from 0.43 g/100 g protein in raw flours of 'Wheat' to 2.82 g/100 g protein in roasted flours of 'Multicereal') than gluten-free infant cereals (from 7.11 g/100 g protein in roasted flours of 'Growth' to 8.64 g/ 100 g in roasted flours of 'Rice and carrot') (Pérez-Conesa et al., 2002).

4. Conclusions

It is widely known that industrial processing affects nutritional value of foods. Among the different components, proteins and nitrogen-containing compounds are those most affected by these treatments, especially thermal processing. The heat and enzymatic treatments, applied during industrial processing to infant cereals, led to a significant increase in NPN and FAA contents, whereas TN and PN contents decreased significantly, NPN content was almost double that of PN in raw flours but, after industrial treatment, the difference increased all the more in commercial gluten-free infant cereals. Therefore, the protein content after industrial processing was clearly decreased, affecting the nutritional value of the infant cereals. However, the industrial treatment might also lead to an enhancement of the nutritional values of infant, cereals by increasing the FAA content, probably through the breakdown of proteins and peptides. The NAANC content of infant cereals, did not show significant variations with the industrial processing, but composition was influenced by the formulation of infant cereals. The only components with significantly increased contents were Cis in 'Wheat' and Sar in 'Multicereal'. The most abundant NAANC in gluten infant cereals were Tau and Asn, while, in gluten-free infant cereals, they were Pet and Cit. The high content of ammonium found in gluten-free infant cereals, in comparison to gluten infant cereals, could be due to the degradation of Tau and Asn in these cereals during industrial processing. Finally, nitrogen content of infant cereals cannot be considered exclusively as protein. Instead special attention should be paid to NPN components in the interpretation of food tables.

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