Original Article

Palatability of Hydrolysates and Other Substitution Formulas for Cow's Milk-Allergic Children: A Comparative Study of Taste, Smell, and Texture Evaluated by Healthy Volunteers

M Pedrosa, CY Pascual, JI Larco, M Martín Esteban

Allergy Department. University Hospital La Paz, Madrid, Spain

Abstract. *Background:* Hydrolyzed formulas used to feed infants with cow's milk-allergy can be classified as soy based, extensively hydrolyzed (casein, whey and mixed), and amino-acid based. Their unsatisfactory taste is reported by parents and physicians.
Objective. The aim of this study was to ascertain the palatability of these formulas in a double-blind taste test.
Materials and Methods: Fifty healthy volunteers performed a randomized-order double-blind test with 12 different milks. The taste, smell, and texture of each formula were evaluated on scales ranging from 1 (worst) to 5 (best). The Pearson correlation coefficient between the peptide weight of each formula and the score obtained for each evaluated attribute was calculated.
Results: The soy formulas and rice formula had the best taste scores, followed by the whey hydrolysates; the mixed hydrolysates and the casein hydrolysates had the lowest taste scores. Individually the most palatable formula was mixed hydrolysate 1, by total score. We found a statistically significant correlation between peptide weight, reflecting the degree of hydrolysis of each formula, and the scores obtained for taste, texture, and overall palatability.
Conclusion: The palatability of formulas is determined by the amount of bitter peptides obtained through hydrolysis. Flavorings and sweeteners may also contribute to palatability. Further studies are needed in order to determine how to modify the organoleptic properties of these products with the purpose of improving their palatability.

Key words: Cow's milk protein allergy. Substitution formulas. Palatability. Bitter peptides. Hydrolysates.

Resumen. *Antecedentes:* Las fórmulas de sustitución utilizadas en la alergia a proteínas de leche de vaca pueden ser: fórmulas de soja, hidrolizados extensos de caseína, hidrolizados de seroproteínas, hidrolizados mixtos y fórmulas elementales. Su sabor poco agradable es referido por padres y médicos.
Objetivo: El objetivo de este estudio es establecer la palatabilidad de estas fórmulas en una cata doble ciego.
Material y Métodos: Cincuenta voluntarios sanos se han sometido a una cata ciega de doce fórmulas diferentes presentadas en orden aleatorio. Sabor, olor y textura de cada fórmula se han evaluado en una escala de 1 (peor) a 5 (mejor). Se ha calculado el coeficiente de correlación de Pearson entre el tamaño de los péptidos presentes en cada fórmula y la puntuación obtenida en los diferentes apartados.
Resultados: Las fórmulas con mejor sabor son las de soja y de arroz, seguidas de hidrolizados séricos y mixtos, y por último el hidrolizado de caseína. Individualmente la fórmula con mejor puntuación total es el hidrolizado mixto 1. Existe una correlación estadísticamente significativa entre el tamaño de los péptidos, es decir, el grado de hidrólisis, y la puntuación obtenida en los apartados sabor, textura y total.

Conclusiones: La palatabilidad de las fórmulas está determinada por el grado de hidrólisis de sus péptidos. También pueden contribuir sustancias como aromatizantes o saborizantes. Se necesitan estudios que modifiquen las características organolépticas de estos productos con el fin de mejorar la palatabilidad.

Palabras clave: Alergia a proteínas de leche de vaca. Fórmulas de sustitución. Palatabilidad. Péptidos amargos. Hidrolizados.

Introduction

Allergy to cow's milk proteins is common in the pediatric population [1], with an incidence of 1.9% in Spain [2]. After a definitive diagnosis, dietary avoidance is mandatory.

Formulas available for allergic children are soy formulas, extensive hydrolysates (whether casein, whey or mixed) and amino acid based formulas [3]. Extensively hydrolyzed formulas provide proteins as peptides of less than 1500 Da. Greater weights are less safe as they have higher antigenic capability [4]. Hydrolysates can be divided into three types, with 100% whey proteins, whey and casein in 60/40 proportions, or 100% casein. Soy formulas offer other treatment options. From an allergenic point of view, soy formulas have nothing to do with cow's milk proteins [3] and are safe, although there is no conclusive evidence supporting indications in the first 6 months of life [4, 5]. The last options are amino-acid based formulas with synthetic amino acids.

The therapeutic options are varied and prescription should be established individually. The main disadvantage of these formulas, however, and the cause of frequent complaints by parents, is rejection by children due to bad taste [5], which is related to the formation of bitter peptides during proteolysis [6]. Many publications report that peptides released by proteolysis have a bitter taste that depends on the size of the peptide and its hydrophobicity, as the alcohol soluble part is the most bitter [7]. Also, bitterness depends on conformational factors, because only part of the peptide interacts with the gustatory receptor [8]. Some authors state that the 193-201 fraction of beta-casein is responsible for the bitterness of hydrolysates [9]. Others assert that bitterness depends on the proteolytic enzyme used [10]. Peptides obtained by proteolysis with exopeptidases taste better because this enzyme hydrolyses hydrophobic peptides [11].

The aim of this study was to determine the palatability of hydrolysates used in the treatment of cow's milk protein allergy and compare palatability scores, as we believe this is a very poorly studied subject. Taste, smell, and texture were evaluated in each formula.

Material and Methods

We carried out a double-blind test of 12 different formulas presented in randomized order. In the study we included semi-skimmed milk, a nonhydrolyzed initiation formula, a partially hydrolyzed formula, 2 casein hydrolysates, 2 whey hydrolysates, and 2 mixed casein-whey hydrolysates. Soy formulas (hydrolyzed and nonhydrolyzed) and a rice hydrolysate were also tested. The compositions of the formulas included in the study are summarized in Table 1.

Fifty healthy volunteers (mean age 34.4 years; range, 25–57 years) were recruited among staff of our hospital and informed of the aims of the study; they freely accepted to participate. Nine (18%) were male and 3 (6%) were smokers. One volunteer was excluded because he suffered from anosmia. As the authors are not aware of any study revealing conclusive evidence of differences in taste or smell perception between children and adults, children were not included in the study [12, 13].

Fifteen percent reconstituted formulas were tested. Aliquots of 10 mL of each formula were offered to volunteers. The test was performed at room temperature (12°C – 22°C). Formulas were first smelled and then tasted; the subject evaluated smell, taste, and texture on scales from 1 (worst) to 5 (best). A total score for each formula was obtained as the sum of the scores obtained in the 3 different sections. Scores were then compared with a Student *t* test. Three-fold mouth washing was performed between each sample. The relationship between the size of peptides and scores was calculated based on the slope of the first segment of the distribution line of the molecular weight of the peptides. Afterwards, the Pearson correlation coefficient (*r*) was used to estimate the correlation between peptide molecular weight and palatability scores. Statistical analysis was performed with the SPSS statistical package version 11.0 for Windows.

Results

The mean scores obtained for each formula are shown in Table 2.

Every substitution formula showed statistically significant differences when compared to cow's milk, in taste ($P < .001$), texture ($P < .05$), smell ($P < .001$) and total score ($P < .001$). The only exception was mixed hydrolysate 1, which did not show a significant difference in smell.

Compared to the initiation formula, all the hydrolysates showed significant differences in taste ($P < .05$) except soy formula. Concerning texture, formulas were similar to the initiation formula, except casein hydrolysate 1, whey hydrolysate 1 and mixed hydrolysate 2, which showed significant differences ($P < .05$). Differences in smell were significant ($P < .001$) in the cases of soy hydrolysate,

Table 1. Characteristics of Hydrolysates*

Hydrolysate	Energy, kcal/100 g. and Caloric Distribution, P/L/C	Proteins, g/100 g	Lipids, g/100 g	Carbohydrates, g/100 g
Partially skimmed cow's milk	45	3.15	1.55	4.65
Initiation formula	74.4	1.56	3.86 L/LN 5.8	8.33 Lactose, 8.07
Partial hydrolysate	76.5	1.73 Cs/S 0/100 Peptides: 140-600 Da: 19% 600-2500 Da: 52.6% 2500-5000 Da: 22.5% >5000 Da: 6.1%	L 0.57 Vegetable 97%	8.66 Lactose, 6.06
Mixed 1	70.2 12/38/50	1.97 Cs/S 40/60 Peptides: <300 Da: 13.5% 300-1400 Da: 68.5% 1400-5700 Da: 18%	3.03 Vegetable MCT 0.66 L/LN 14	8.76 DTM, 7.86 Potato starch, 0.9
Mixed 2	77.7 10/49/41	2.03 Cs/S 40/60 Peptides: <1000 Da: 89%	4.2 Vegetable MCT 0.63 L/LN 9.7	7.95 DTM, 7.47 Potato starch, 0.48
Whey 1	73.35	2.48 Peptides: <500 Da: 63-74% 500-2500 Da: 12%-19% 2500-6000 Da: 11%-15%	3.6 Vegetable MCT 50% L/LN 19	7.76 DTM, 6.74 Potato starch, 0.9 Lactose, 0.12
Whey 2	78.3 10/50%40	1.86 Peptides <1500 Da: 86% 1500-3000 Da: 10% >3000 Da: 4%	4.28 Vegetable L/LN 5.5	8.12 DTM, 5.03 Lactose, 3.08
Casein 1	75 11/45/44	2.1 Peptides: <500 Da: 60.4% <1000 Da: 95.4% <3000 Da: 100%	3.75 Vegetable L/LN 10.4	8.25 Glucose syrup, 80% Corn starch, 20%
Casein 2	77.25 10/47/43	1.86 Peptides: <500 Da: 39.9% <1000 Da: 81% <3500 Da: 100%	4.02 Vegetable L/LN 10	8.4 Glucose syrup
Soy hydrolysate	74.55 11/44/45	2.03 Soy and pork collagen hydrolysate Peptides: <1000 Da: 71% <2000 Da: 89% <5000 Da: 98.9%	3.6 Vegetable L/LN 5.5	8.55 DTM 6.59 Corn starch, 1.41
Soy formula	77.55 10/49/41	2.06	4.05 Vegetable L/LN 6.1	7.88 Sucrose Corn syrup
Rice formula	71	2	3.1	8.6 DTM, 6.9 Corn starch, 1.7

*P indicates proteins; L, lipids; C, carbohydrates; L/LN, linoleic acid/linolenic acid; ratio of casien to serum proteins; DTM, dextrin maltose; MCT, medium chain triglycerides.

Table 2. Slope of the First Segment of the Peptide Molecular Weight Distribution Line and Palatability Scores for Formulas*

	Slope	Taste	Smell	Texture	Total	*P* Comparison	
						With CM	With IF
Cow's milk		4.10	3.74	4.06	11.9		
Initiation formula		3.44	3.22	3.22	9.88		
Partial hydrolysate		2.78	3.22	3.54	9.54	<.001	NS
Mixed hydrolysate 1	58.57	2.22	3.68	3.18	9.08	<.001	NS
Whey hydrolysate 2	57.30	2.66	2.82	3.52	9.00	<.001	NS
Casein hydrolysate 2	81	1.86	2.14	2.90	6.90	<.001	<.001
Whey hydrolysate 1	137	1.52	2.26	2.60	6.38	<.001	<.001
Casein hydrolysate 1	120.80	1.32	2.02	2.40	5.74	<.001	<.001
Mixed hydrolysate 2	89	1.38	1.62	2.28	5.28	<.001	<.001
Soy formula		2.80	2.70	2.94	8.44	<.001	NS
Rice hydrolysate		2.56	2.64	2.74	7.94	<.001	<.05
Soy hydrolysate	71	2.40	2.40	2.82	7.62	<.001	<.001

*CM indicates cow's milk; IF, initiation formula; NS: not statistically significant.

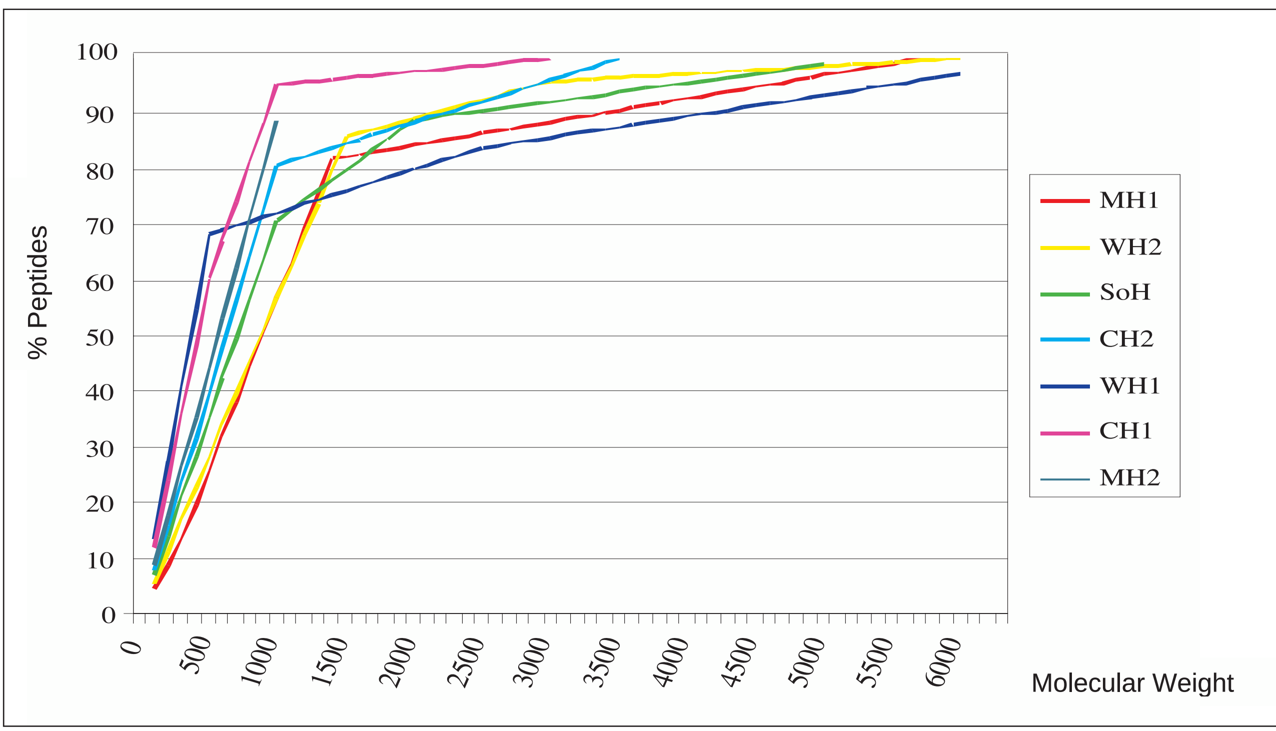

Figure 1. Distribution lines of peptides' molecular weights (Da). The higher the percentage of small peptides, the greater will be the degree of hydrolysis and the steeper the slope of the line. MH indicates mixed hydrolysate; WH, whey; SoH, soy hydrolysate; CH, casein hydrolysate.

whey hydrolysate 1, mixed hydrolysate 2 and both casein hydrolysates. Soy formula, whey hydrolysate 2 and mixed hydrolysate 1 were very similar to the initiation formula in terms of the total score.

Between the 2 mixed hydrolysates, the only differences were in smell ($P < .001$); no statistically significant differences were found in taste or texture. The 2 whey hydrolysates differed in taste and texture ($P < .001$). Smell showed no statistically significant difference. The 2 casein hydrolysates were similar to each other, as were the soy formulas.

Figure 1 shows the distribution of peptides by molecular weight for each formula, according to data obtained from the supplier. The slope of the first segment

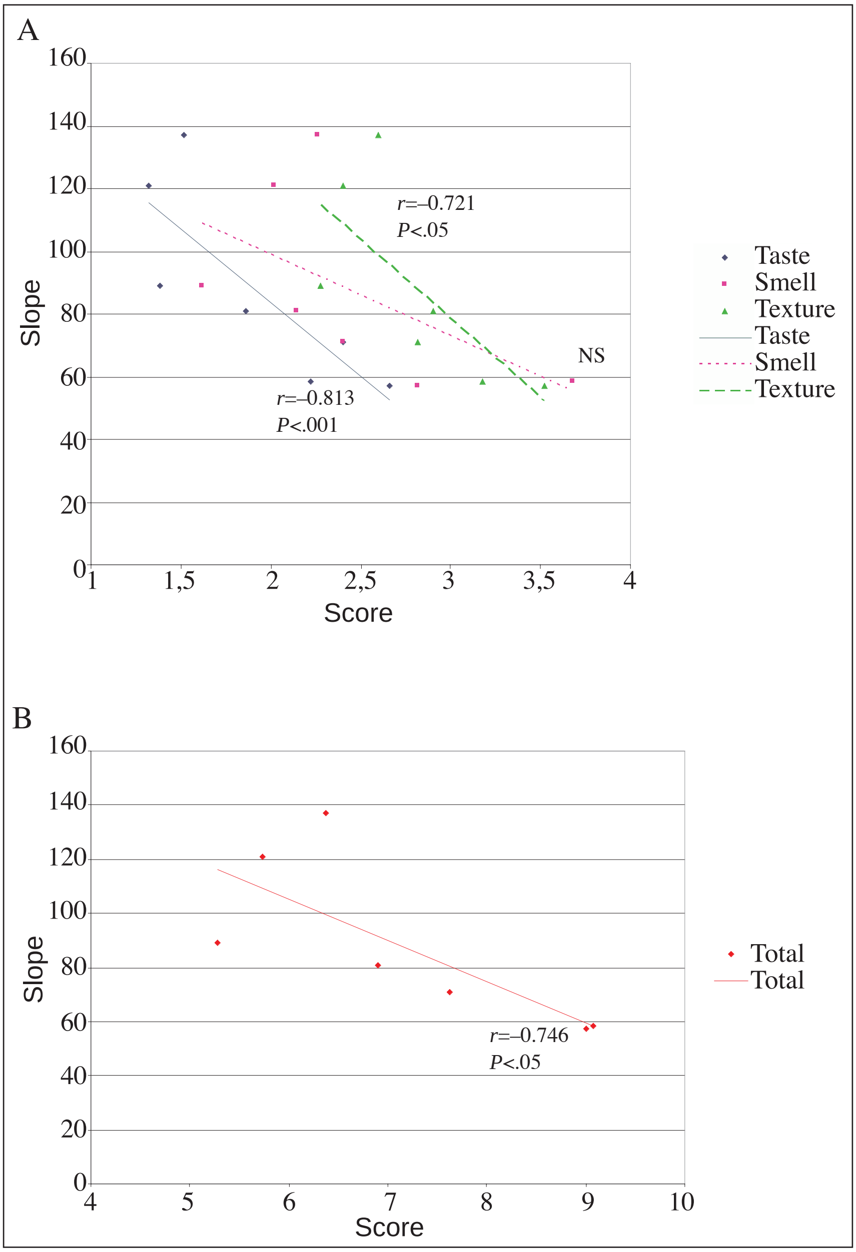

Figure 2. Correlation (Pearson coefficient, *r*) between slope of the first segment of the peptide molecular weight distribution line and individual taste, smell, and texture scores (A) and the overall palatability score (B), and the slope of the first segment of the distribution line of peptides by molecular weight. The tendency line is also shown.

of the distribution line was steeper in accordance with a lower peptide weight, indicating a greater degree of hydrolysis. Figure 1 shows that the formulas with the best scores (mixed hydrolysate 1 and whey hydrolysate 2) had less steep slopes, meaning their peptides are smaller.

The slope of the first segment of each distribution line was calculated (Table 2) and compared with the score. Steeper slopes, that is, a higher degree of hydrolysis, correlated with lower scores and the correlation was more marked for the taste score. Figure 2 shows the Pearson correlation coefficient for the relation between slopes and scores. There was good correlation between slope and scores for taste, texture and the total with a high level of significance ($P < .05$ for total and texture and $P < .001$ for taste).

Discussion

This is the first study to evaluate the palatability of the substitution formulas available for cow's milk protein-allergic children. We have considered it appropriate to evaluate palatability as the sum of taste, smell, and texture because all of them contribute decisively to the gustatory sensation.

The taste score results for formulas grouped by type showed that partial hydrolysates taste better than the rest. Soy formulas tasted better than whey hydrolysates, which were better than mixed, and mixed were better than casein formulas. This difference could be explained by the existence of certain casein fractions obtained after hydrolysis [9] which are not obtained in the hydrolysis of other formulas. The fact that formulas of the same type obtained different scores means that, apart from hydrolysis and bitter peptide release, there must be other factors that influence the taste, such as the addition of sweeteners or flavorings, such as vanillin, that could contribute to the gustatory sensation. Differences between the 2 soy formulas, on the other hand, may be due to the fact that one of them is hydrolyzed.

The total score was very similar to the taste score for many of the formulas because smell and texture scores showed a very similar distribution. The most remarkable exception was mixed hydrolysate 1, which had a much higher total score than taste score due to its higher smell score.

If we compare scores obtained by each hydrolysate with the slope of the distribution line for peptide molecular weight, there seems to be a relation between total score and the degree of hydrolysis. This is more noticeable in the first segment of the line. The Pearson coefficient between slope and the total score and especially the taste score showed a statistically significant correlation. This correlation is consistent with findings that the degree of hydrolysis determines the release of bitter peptides that contribute to bad taste [7].

We can conclude that mixed hydrolysates are the formulas with the best taste in general, although, individually, the formulas with higher total scores are mixed hydrolysate 1 and whey hydrolysate 2. Both formulas have a very similar score to that obtained by the initiation formula and the partial hydrolysate. Further studies should be carried out in order to modify organoleptic properties of hydrolysates on behalf of better taste and texture.

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María Pedrosa Delgado

Servicio de Alergología
Hospital Universitario La Paz
Pº Castellana, 261
28046 Madrid, Spain
E-mail: maria_pedrosa@yahoo.es

