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Full Length Research Paper

Low phenylalanine pasta

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Four formulas were prepared for production of low phenylalanine pasta suitable for phenylketonuria (PKU) patients. The formulas were based on partial replacement of wheat flour with different levels of corn starch. Pectin and carboxymethylcellulose (CMC) were added as a texture modifier. Chemical composition, amino acids, dough characteristics, color attributes, cooking quality, electron microscopic examination and sensory evaluation of pasta were investigated. The obtained results revealed that pasta samples had lower protein and phenylalanine content than control as a result of replacing wheat flour using corn starch. Phenylalanine and protein content of formula "D" which contains 66 g corn starch, 30 g wheat flour, 2 g pectin and 2 g CMC reduced by 68 and 70%, respectively, compared with control. Viscoamylograph and falling number parameters were significantly affected. The cooking loss of pasta ranged from 10.60 to 22.50% and was significantly higher than that of the control, but adding hydrocolloids (pectin and CMC) diminished cooking loss. Sensory characteristics of pasta indicated that all formulas were acceptable. Formula "D" which has the lowest phenylalanine content seems to be superior for PKU patients.

Key words: Pasta, wheat flour, corn starch, pectin, carboxymethylcellulose, phenylketonuria, amino acids, microstructure, sensory evaluation.

INTRODUCTION

If the present trend in consumer awareness of nutrition continues, the need for special dietary foods designed to correct nutritional imbalance is expected to increase in the coming years. However, this need cannot be met unless special dietary foods with sensory properties similar to those of traditional foods are developed. The quantity of protein and its amino acids in wheat flour have a great effect on patients who are suffering from phenyketonuria (PKU) (Dwivedi, 1986). PKU is an autossomal recessive disease caused by phenylalanine-hydroxylase (PAH) deficiency. The deficiency of this enzyme leads to the accumulation of phenylalanine in tissues and plasma of patients (Hendriksz and Walter, 2004; Sirtori et al., 2005). Minimal requirements of phenylalanine (9.1 $mg.kg^{-1}d^{-1}$) are needed to meet normal growth and development. Excess phenylalanine (that not required for protein anabolism) is mainly metabolized in the liver by PAH. In the United Kingdom, a system of protein exchanges is used, with approximately each 1 g natural protein representing a phenylalanine load of 50 mg. Most children with classic PKU can tolerate less than 500 mg phenylalanine or 10 g protein exchanges in 24 h (Hendriksz and Walter, 2004; Pencharz et al., 2007).

Low phenylalanine foods may be prepared by selecting ingredients that are low in protein, i.e. starch, cellulose derivatives and gums. The functional effects of hydrocolloids stem from its ability to modify dough or batter rheology (or handling) and keeping quality of finished products (Guarda et al., 2004; Ribotta et al., 2005; Lazaridou et al., 2007; Shittu et al., 2009).

Pasta has long been a favorite food of Chinese and Mediterranean civilization and is currently consumed and appreciated worldwide. It is simple to prepare, easy to transport and has excellent storage properties. Pasta is recognized as low in sodium and fat, with no cholesterol, and as a rich source of complex carbohydrates (Giese, 1992). In spite of these facts, a 2 oz serving of dry pasta

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Table 1. Formula of low phenylalanine pasta.

Ingredients* (%)	Control	Pasta formulas			
		A	B	C	D
Wheat flour (72% ext.)	100	60	50	40	30
Corn starch	-	36	46	56	66
Pectin	-	2	2	2	2
CMC	-	2	2	2	2

*Water was added to all formulas as needed. CMC = carboxymethylcelullose.

provides about 10% protein, which is over the recommended level for a patient oriented on a low protein diet *i.e.* renal failure and hepatic diseases. So dilution of protein with non-proteinaceous materials should enable these patients to benefit from this nutritious product (Caperuto et al., 2000; Gianibelli et al., 2005).

Therefore the purpose of this study was to formulate different low phenylalanine pasta dough formulations. Chosen materials were wheat flour, corn starch, pectin and carboxymethylcellulose (CMC) to produce low phenylalanine pasta. Chemical composition, amino acids content, rheological properties, cooking quality, sensory evaluation and electron microscopic examination of pasta were studied.

MATERIALS AND METHODS

Wheat flour (72% extraction) was obtained from North Cairo Flour Mill Company, Egypt. Pectin and CMC (Food Grade) were purchased from Sigma Company, Germany. Corn starch (Food Grade), was obtained from the Starch and Glucose Manufacturing, Tora, Cairo, Egypt.

Formulation of low phenylalanine pasta

Wheat flour (72% extraction) was well blended with corn starch. Pectin and CMC were added to wheat flour/corn starch blends. Four blends were prepared and control sample was made with 100% wheat flour for comparison. Table 1 summarizes the composition of the blends.

Processing of pasta

Pasta samples were processed using Pasta Matic 1000 Simac Machine Corporation, Millano, Italy, under extrusion conditions as described by Hallabo et al. (1985). The pasta samples were laid in a single layer on cloth-lined trays. Samples were dried at 70°C for 10 h according to Berglund et al. (1987). Dried samples were broken into 8-10 cm pieces and placed in sealed jars until analysis.

Cooking quality of pasta

Cooked weight (g), as a measure of the degree of pasta hydration, of 10 g dry pasta was recorded as described in American Association of Cereal Chemists (AACC) (1983) method No 16-50. The pasta was considered cooked when the observed white core had disappeared after the pasta was pressed between two plexi glass plates.

Cooking loss, weight of total solids expressed as percent (%), was measured by evaporating the pasta cooking water to dryness in an oven at 100°C as described in AACC (1983) method No 16-50.

Volume increase of cooked pasta was determined as an indicator of swelling. Petroleum naphthan was used in measuring sample volume.

Chemical analysis

Moisture, ash, protein, fat and crude fiber contents were determined according to the methods outlined in Association of Official Analytical Chemists (AOAC) (2000) Carbohydrates were calculated by difference according to the following equation:

Total carbohydrate = 100- (protein + fat + crude fiber + ash).

Rheological properties

Rheological properties of dough were recorded by a Brabender viscoamylograph (C.W. Brabended Instruments, South Hackensack, NJ) according to AACC (1983). Falling number test was carried out according to AACC (1983).

Amino acids content

The dry pasta samples were hydrolyzed in sealed tubes with 10 mL HCl (6N); 24 h; 110°C in a sandy bath. The hydrolyzed samples were filtered through a 0.45 µm nylon filter, evaporated at 40°C in a rotary evaporator and then dissolved with 1 ml deionized water and evaporated once again in order to remove the traces of the acid. The residue was reconstituted in 1 ml of deionized water then 20 µL was injected into the amino acid analyzer (Eppendorf LC3000, Germany) for the determination of the amino acid composition of each sample. The amino acids were separated on a cation exchanger resin column (150 mm x 2.6 mm i.d., No. 2619 resin) using citrate buffer at pH 2.2, a column temperature of 53°C, a flow rate of 0.225 mL/min and a postcolumn reaction with ninhydrin (0.3 mL/min ninhydrin flow rate) followed by a photometric detection at 570 nm (Li et al., 2006). Tryptophan was not determined.

Color quality of pasta

The color of dry pasta samples was measured using a spectro-colorimeter with the CIE color scale (Hunter, Lab scan XE). This instrument was standardized against the white tile of Hunter Lab color standard (LX No.16379): X= 77.26, Y= 81.94 and Z= 88.14. The L, a and b values were reported. Total color difference (ΔE) was calculated as:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Table 2. Chemical composition of raw materials and their mixtures (% on dry weight basis).

Sample	Protein	Ash	fat	fiber	CHO
Wheat flour	9.60±0.12	0.66±0.08	1.10±0.09	0.78±0.07	87.86±0.22
Corn starch	0.50±0.06	0.28±0.01	0.30±0.01	0.22±0.01	98.70±0.35
Flour mixtures*					
A	5.74±0.13	0.50±0.04	0.80±0.03	0.57±0.08	92.39±0.32
B	4.82±0.16	0.46±0.04	0.70±0.04	0.50±0.06	93.92±0.28
C	3.85±0.11	0.42±0.02	0.63±0.02	0.43±0.03	94.67±0.43
D	2.90±0.08	0.40±0.03	0.54±0.03	0.37±0.04	95.79±0.38

*For dough formula (Table 1).

Table 3. Viscoamylograph parameters of dough formulas.

Sample	Transition temp. (°C)	Maximum viscosity (BU)	Temp. at maximum viscosity (°C)	Breakdown viscosity (BU)	Setback viscosity (BU)
Control	65	400	86	190	500
Dough formulas*					
A	66	2200	94	1750	2500
B	68	2400	94	1900	2800
C	69	2600	94	2050	3100
D	70	2900	94	2100	3450

*For dough formula (Table 1). BU = Brabender Unit.

Sensory evaluation of pasta

Appearance, color, flavor, tenderness and stickiness of cooked pasta were organoleptically evaluated by ten panelists as described by Hallabo et al. (1985).

Electron microscopy scanning

A cross-section of uncooked pasta was coated with gold-palladium and the internal structure was examined by a Jeol scanning electron microscope type JXA 840A, Japan. Electron Microscopy Scanning of these samples were taken at magnifications of 500×.

Statistical analysis

The obtained results were statistically analyzed by analysis of variance (ANOVA) and least significant difference (LSD) was calculated according to McClave and Benson (1991) using HDSS program.

RESULTS AND DISCUSSION

Chemical composition of raw materials and their mixtures

Proximate composition of raw materials and their mixtures are summarized in Table 2. Corn starch showed lower protein content and higher carbohydrates than wheat flour, 0.50 and 98.70% respectively. So, addition of corn starch to wheat flour decreased the protein content of the produced mixtures. For instance, the protein content of wheat flour that was 9.60% reduced to 2.90% for formula "D". Same findings were noticed when ash, fat and fiber were considered. Such results were in agreement with those obtained by El-Bardeny and Moustafa (1993) and Yaseen and Ahmed (1999).

Pasta dough rheology

Pasta dough formulas were rheologically evaluated by viscoamylograph for heat of transition, maximum viscosity, temperature at maximum viscosity, break down viscosity and set-back viscosity as presented in Table 3. Results showed that control sample had 65°C, 400 BU, 86°C, 190 BU and 500 BU for heat of transition, maximum viscosity, temperature at maximum viscosity, break down viscosity and set-back viscosity, respectively. Replacing wheat flour using corn starch at the different levels and adding hydrocolloids in all formulas gradually increased all measured parameters (66-70°C), (2200-2900 BU), (1750-2100 BU) and (2500-3450 BU) for heat of transition, maximum viscosity, break down viscosity and set-back viscosity. This might be attributed to the higher content of starch in the blends and starch characteristics of corn compared to those of wheat starch. The gelatinization temperature of wheat starch (54-62°C) is lower than that of corn starch (60-71°C) as reported by

Table 4. Falling number parameters of pasta dough formulas*.

Sample	Falling number (sec)	Falling time (s)	Liquefaction number
Control	300	240	24.00
Dough formulas**			
A	395	335	17.40
B	410	350	16.67
C	425	365	16.00
D	430	370	15.79

*Data are average of triplicate analysis. ** For dough formula (Table 1).

Table 5. Cooking quality of prepared pasta*.

Sample	Weight increase (%)	Volume increase (%)	Cooking loss (%)
Control	200 ± 3	250 ± 5	10.60±1.2
Dough formulas**			
A	179 ± 4	240 ± 3	18.00±0.8
B	162 ± 2	230 ± 5	19.74±0.9
C	155 ±3	225 ± 3	21.00±1.5
D	150 ± 2	220 ± 2	22.00±1.7

*Data are average of triplicate analysis. ** For dough formula (Table 1).

Colonna and Mercier (1985). Since corn starch granules are more rigid than wheat starch granules, it requires more heat energy to achieve complete swelling. Shuey (1975) reported that, higher amylogram values indicate less amylase activity and conversely lower amylogram values indicate higher activity, extremely low values or high activity will cause slackening of the dough, especially during fermentation. The amount of slackening depends on the starch damage of the flour.

Lazaridou et al. (2007) studied the effect of hydrocolloids on dough rheology in gluten-free formulations based on rice flour, corn starch, and sodium caseinate. The hydrocolloids added at 1% and 2% (rice flour basis) were pectin, CMC, agarose, xanthan and oat β-glucan. The study on rheological behavior of the doughs containing hydrocolloids, performed by farinograph and rheometry, showed that xanthan had the most pronounced effect on viscoelastic properties yielding strengthened doughs. Moreover, among the preparations supplemented with hydrocolloids the elasticity and resistance to deformation of dough, as determined by oscillatory and creep measurements, followed the order of xanthan > CMC > pectin > agarose > β-glucan.

Falling number

The falling number (FN) determination is commonly used to estimate α-amylase activity in wheat meal and flour (Finney, 2001). FN, falling time and liquefaction number of all dough formulas are shown in Table 4. The results indicated that FN and falling time increased while liquefaction number decreased for all formulas compared with control. For instance, FN of formula "D" was 430 s with about 43% increase than the control dough. Liquefaction number indicated a value of 24 for the control sample the reduction was about 34% for formula "D". The increasing of FN is mainly due to that all formulas contain more starch. This means that addition of corn starch increased the viscosity. Also the effect of hydrocolloids (pectin and CMC) was similar to that of starch. Hydrocolloids modified food texture and consequently increased viscosity. Similar findings were also observed by Boyacioglu and D'Appolonia (1994), they reported that lower levels of α-amylase enzymes means higher FN value.

As shown in the same table, as FN increased, falling time increased while liquefaction number decreased this trend was observed in all tested samples. The results obtained by FN test confirmed that obtained by viscoamylograph test.

Cooking quality of pasta

Results in Table 5 reflect the cooking quality of processed pasta. Over all, there were large differences in the weight of the cooked samples. Control sample gained about 2 times of its weight when cooked in water, while starch containing samples ranged from 1.79 to 1.50 times of their weight. The ideal expected cooked weight of semolina spaghetti is about three times of the dry weight

Table 6. Color quality of pasta.

Sample	Lightness "L"	Redness "a"	Yellowness "b"	ΔE
Control	70.02	2.95	18.98	0.00
Dough formulas**				
A	75.56	2.64	17.44	5.76
B	77.45	2.46	15.67	8.15
C	79.00	2.21	14.00	10.30
D	81.80	2.08	12.30	13.57

*Data are average of triplicate analysis. ** For dough formula (Table 1).

Table 7. Sensory evaluation of pasta.

Sample	Appearance (10)	Color (10)	Flavor (10)	Tenderness (10)	Stickiness (10)
Control	8.75[a]	8.87[a]	9.12[a]	9.00[a]	8.87[a]
Dough formulas*					
A	7.87[ab]	6.87[b]	7.87[bc]	7.73[bc]	7.87[ab]
B	6.87[bc]	6.00[bc]	7.50[c]	6.75[cd]	6.87[bc]
C	6.12[cd]	5.87[bc]	7.00[c]	5.87[de]	6.12[cd]
D	5.62[d]	5.12[c]	5.75[d]	5.37[e]	5.62[d]
LSD	1.120	1.308	1.132	1.082	1.155

*For dough formula (Table 1).
There is no significant difference between two means (within the same property) designed by the same letter ($p<0.05$).

(Dick and Youngs, 1988). The volume of the control pasta increased 2.5 folds after cooking, while it ranged from 2.4 to 2.2 fold for all formulas. These decrements in weight and volume of pasta may be due to the increase in cooking loss and dilution of gluten network where it became weak and had less able to absorb water.

Regarding the cooking loss (solids lost into cooking water) Table 5 shows that in all cases, the cooking loss of all formulas was higher compared to the control sample. The undesirable effect on cooking loss may be due to the dilution of the gluten by increasing the substitution levels with corn starch. Noteworthy, increasing the addition of hydrocolloids or gums diminished the cooking loss. This could be explained by the binding ability of gum added. Caperuto et al. (2000) reported that gluten-free pastas such as corn products normally exhibit large leaching value on cooking, of the order of 200-250 g/kg while, soft wheat spaghetti show losses of solids between 110 and 170 g/kg. Also, Abcassis et al. (1989) proposed that addition of maize flour to durum flour at the level of 66% increased the cooking loss from 7% for durum pasta to 14% for maize-durum pasta. The weight of 100 g uncooked durum pasta increased to 320 g after cooking while this increment decreased to 280 g for maize-durum pasta.

Pasta color evaluation

Color of dry pasta is an important quality factor for consumers. Color quality of uncooked pasta samples are shown in Table 6. Pasta containing corn starch was lighter in color, the lightness (L values) increased with increasing the level of added corn starch compared to control sample. All formulas were generally 5-12 units more than control. Significant decreases were found for "b" values. Greatest decrease was caused by the higher level of substitution. For example, value of "b" of control was found to be 18.98, while it was 12.30 for the formula "D" (with about 35% reduction).

The highest color differences "ΔE" was recorded for formula "D" followed by formula "C". However, these differences in color between control sample and those formulas which containing corn starch could be minimized by applying colored seasoning during processing. These findings are in agreement with those obtained by Yaseen and Ahmed (1999).

Sensory evaluation of pasta

Results of the sensory evaluation of pasta are shown in Table 7. All formulas were rated lower for appearance, color, flavor, tenderness and stickiness than the control sample. Taste and palatability of produced pasta were acceptable. Significant differences at ($P<0.05$) were observed within all pasta samples and between the control for all sensory characteristics. The color of pasta changed and became lighter by increasing levels of corn starch in all formulas.

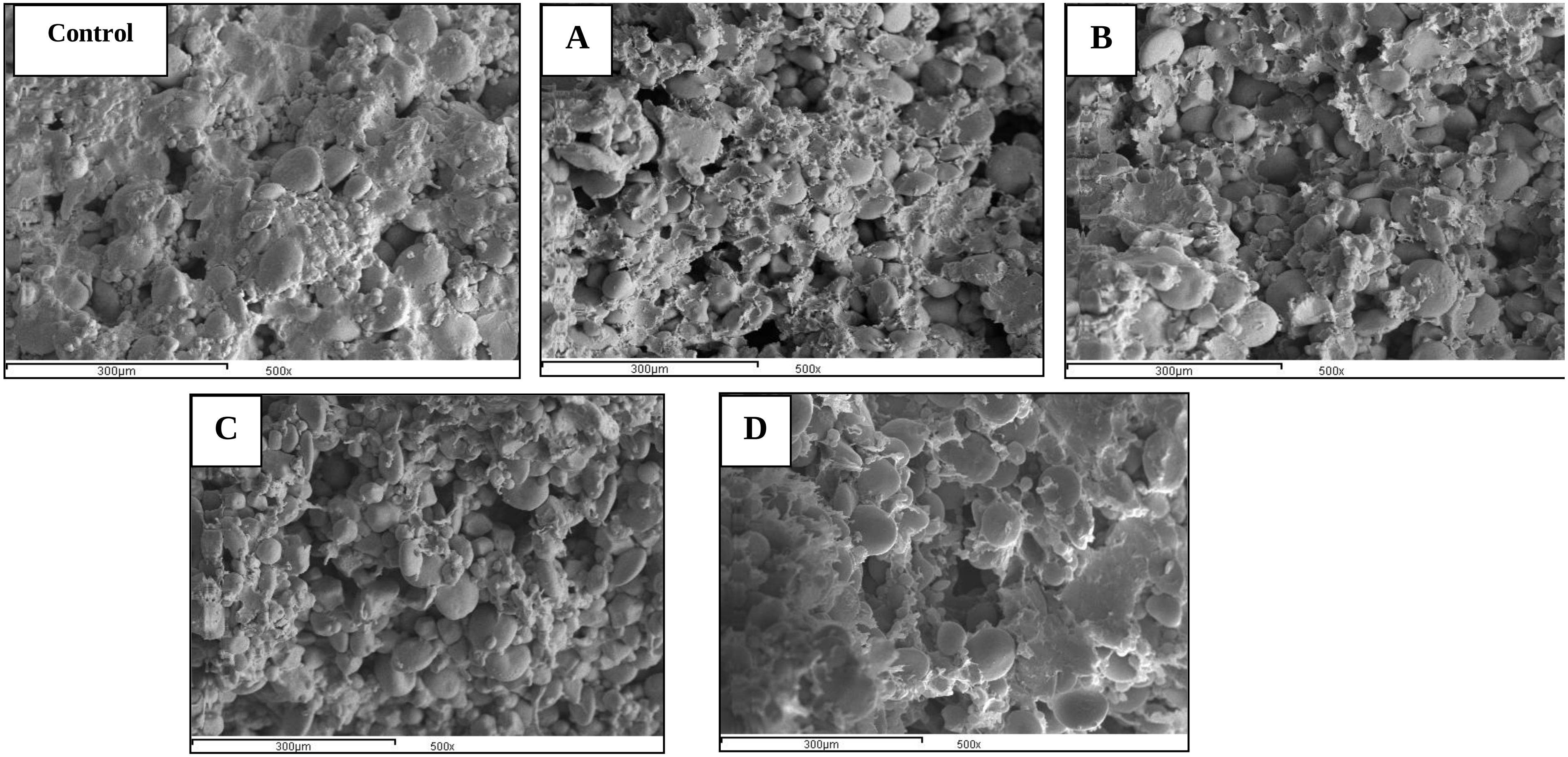

Figure 1. Scanning electron photomicrograph of pasta.

This was further supported by measuring the total color difference using Hunter Lab (Table 6). A significant decreased score for clumping of spaghetti containing 60% substitution of starch, at this level a clumpy texture was detected by panelists; gum addition did not overcome this property. Also, the color score of spaghetti containing 60% starch was the most dissimilar, whereas 20% of starch substitution gave the least color difference (Yaseen and Ahmed, 1999).

Microstructure of pasta

Electron microscopy scanning at magnification of 500X was used to follow the morphological changes in the internal structure of pasta samples and the results are presented in Figure 1. Electron microscopy scanning results showed that control sample was characterized by a smooth, continuous layer of protein coating the air cells. Starch granules with smaller diameter with distinctive large lenticular-shaped and smaller spherical were enveloped in the gluten which observed on the

Table 8. Amino acid composition and protein content of dry pasta (g/100g dry sample).

Amino acids	Control	Pasta formulas			
		A	B	C	D
Essential amino acids (EAA)					
Threonine	0.24	0.14	0.12	0.09	0.07
Valine	0.30	0.18	0.15	0.12	0.09
Methionine	0.16	0.09	0.08	0.06	0.05
Isoleucine	0.25	0.15	0.13	0.10	0.08
Leucine	0.53	0.32	0.27	0.21	0.16
Phenylalanine	0.38	0.23	0.19	0.15	0.12
Histidine	0.24	0.14	0.12	0.09	0.07
Lysine	0.19	0.11	0.10	0.08	0.06
Total EAA	2.29	1.36	1.16	0.90	0.70
Non essential amino acids (NEAA)					
Aspartic acid	0.38	0.23	0.19	0.15	0.12
Serine	0.30	0.18	0.14	0.12	0.08
Glutamic acid	2.25	1.34	1.13	0.90	0.69
Proline	0.64	0.38	0.32	0.25	0.19
Glycine	0.28	0.17	0.14	0.11	0.07
Alanine	0.25	0.15	0.12	0.10	0.08
cystine	0.19	0.11	0.09	0.08	0.06
Tyrosine	1.24	074	0.62	0.50	0.38
Arginine	1.05	0.63	0.53	0.42	0.32
Total NEAA	6.59	3.93	3.28	2.63	1.99
Total AA	8.88	5.29	4.44	3.53	2.69
Protein (%)	9.67	5.76	4.85	3.87	2.94

Tryptophan was not determined.

surface of the granules. Incorporation of corn starch into pasta gradually affected its internal structure where the air cell disappeared gradually as the starch level increased and discontinuous protein layers appeared. The starch granules seemed to be larger as it was not homogenous with remnant starch granules.

At high levels of corn starch (formula D) pasta gave areas of compact cells in the center of the rod rather than a lattice-filled structure. The doughs disintegrated and discontinuous gluten particles were observed. This indicates that addition of corn starch made the dough fragile and susceptible to breakage.

Previous observations supported the obtained results of cooking quality. That confirms the strong correlation between pasta cooking loss and distribution of the cells in the pasta samples.

Amino acids composition and protein content of pasta

Amino acids and protein content of all pasta samples are shown in Table 8. The results indicated that all formulas of pasta had lower total amino acids compared with control. The total amino acids of the A, B, C and D formulas were 5.29, 4.44, 3.53 and 2.69 g/100g dry sample constituting about 40, 50, 60 and 70% reduction compared to control pasta, respectively. Same findings were noticed when essential and non essential amino acids were considered. The tested samples showed higher values of glutamic acid, tyrosine, arginine and proline whereas the lower amino acid values were methionine, cystine and lysine.

The results also indicated that all formulated pasta were characterized by lowering phenylalanine content by 39, 50, 61 and 68% for formulas A, B, C and D compared with control, respectively. On the other hand all formulated samples were found to contain the lowest amounts of protein compared with control pasta. For instance, protein content of formulas A, B, C and D was 5.76, 4.85, 3.87 and 2.94%, respectively while it was 9.67% for the control sample.

From the previous results, it could be concluded that, all suggested formulas (A, B, C and D) are suitable for production of well tolerated and palatable low phenylalanine pasta, but formula "D" had superior acceptability for PKU patients regarding phenylalanine requirements.

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