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Quality Characteristics Improvement of Low Phenylalanine Pasta

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> DW phenylalanine pasta was produced for phenylketonuria (PKU) patients using degliadin wheat flour mixed with pectin, gum arabic, and carboxymethylcellulose (CMC) at the levels of 1, 2, and 3% (W/W). Gliadin, the phenylalanine rich fraction, was separated using an alcohol procedure to minimize the phenylalanine content pasta. Chemical composition, amino acids content, pasting properties, organoleptic characteristics, cooking quality, and pasta microstructure were investigated. Results indicated that the reduction in phenylalanine content of pasta manufactured using de-gliadin wheat flour was 42.6% compared to the control pasta. But on the other hand, de-gliadin dough and pasta were characterized with poor rheological properties and cooking quality, respectively. The addition of hydrocolloids pronouncedly improved these properties. These variations were further explained through the microstructure examination of pasta, which revealed disintegrated and discontinuous gluten particles in degliadin pasta. Acceptable low phenylalanine pasta could be manufactured using de-gliadin wheat flour containing 2% pectin or 3% CMC for PKU patients.

Keywords: Phenylalanine, Pasta, PKU, Rheology, Hydrocolloids, Microstructure.

Introduction

Pasta is a convenient, nutritious, and palatable wheat-based product that gained high popularity worldwide (Petitot et al., 2009). According to the Italian definition, pasta is a product that is mainly obtained through extrusion or lamination of durum wheat semolina dough. The processed dough could be directly cooked or subjected to further drying steps (Carini et al., 2010). In pasta processing, gluten plays a vital role, since it is the main responsible for the firm structure formation, adhesiveness and cooking loss reduction, and al dente chewability of pasta (Sozer, 2009). However, some people with specific genetic disorders like celiac and phenylketonuria (PKU) diseases lack the ability to metabolize gluten proteins (Dwivedi, 1986).

In the case of PKU patients, minimum

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phenylalanine quantities (9.1 mg/kg/d) are required to meet the normal growth and development. Upon consumption of excess phenylalanine, it accumulates in patient's plasma and tissues causing mental retardation due to the deficiency of phenylalanine-hydroxylase (PAH), which is mainly responsible for phenylalanine metabolization in the liver (Hendriksz & Walter, 2004; Pencharz et al., 2007; Sirtori et al., 2005). So, the only effective PKU treatment is to reduce the amount of phenylalanine in the diet (Courtney-Martin et al., 2002). This means that foods containing a high proportion of phenylalanine have to be avoided, including meat, milk, beans, cereals, and their products.

Gluten represents about 85% of wheat proteins and consists of gliadin and glutenin fractions (Spurway, 1988). Phenylalanine content

of glutenin and gliadin was found to be 4.36 and 5.75 g/100 g protein respectively (Byers et al., 1983). The separation of gliadin from wheat flour was able to reduce the phenylalanine content of the final product by 43.2% (Mohsen et al., 2010). Hydrocolloids i.e. guar gum, gum arabic, alginates, and CMC could be used to improve firmness, mouthfeel, and to increase the rehydration rate of pasta upon cooking (Mandala et al., 2007; Rojas et al., 1998; Shalini & Laxmi, 2007).

The incidence of PKU is approximately 1:10000 in many populations. However, it might increase particularly in Ireland and Western Scotland (1:4500). Caucasians in the United States have a PKU incidence of 1:8000 whereas blacks have an incidence of 1:50000 (Hendriksz & Walter, 2004). In Egypt, PKU is the most common metabolic disorder with an incidence of 1:7500 (Effat et al., 2008; Shawky et al., 2006). Therefore, the production of different varieties of low phenylalanine food products in Egypt is very important for consumers who suffer from PKU. Therefore, the present study aimed to produce low phenylalanine pasta using de-gliadin wheat flour supplemented with hydrocolloids and to evaluate the quality characteristics of the processed pasta.

Materials and Methods

Materials

Pectin [Food Grade, (methoxyl content 7%, sulfated ash 4%) was purchased from Sisco Research Laboratories PVT. LTD., India], Gum Arabic (acacia gum) [Food Grade, (ash content more than 4%) was purchased from Fluka Company, Switzerland] and Carboxymethylcellulose (CMC) [Food Grade (potassium 10%, calcium 1.7%, sodium 1%) purchased from Sigma Company, Germany] were used. Wheat flour (72% extraction) was purchased from the local market, Giza, Egypt.

Methods

Sequential extraction of wheat protein fractions

Albumin, globulin, gliadin, and glutelin fractions of wheat protein were sequentially separated using distilled water, 1 *M* NaCl, 55% (v/v) 1-propanol, and 0.05 *M* NaOH, respectively according to the method of Guo & Yao (2006).

Determination of protein and amino acid profiles

Protein content of wheat protein fractions was measured according to AOAC (2000). Acid hydrolysis procedure was used to determine the amino acid profiles according to Li et al. (2006). Briefly, samples were hydrolyzed using HCl (6N),

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filtered, evaporated, and dissolved in deionized water. Amino acids were separated using an amino acid analyzer (Eppendorf LC3000, Germany) on a cation exchanger resin column, citrate buffer as a mobile phase and ninhydrin as a post-column reaction followed by detection at 570 nm.

Preparation of de-gliadin wheat flour

One hundred grams of wheat flour (72% extraction) were extracted twice using 1 L of propanol : water (55 : 45 v/v) for 1 h at room temperature, then centrifuged (Chermle Z320, Germany) at 10,000 g for 20 min. The residues were collected and dried (Ehret GmbH, Germany) overnight at 50°C. The dried residues were ground (Quadrumat Junior Mill, Germany) to obtain the de-gliadin flour. De-gliadin flour was mixed with pectin, gum Arabic, and CMC at the levels of 1, 2, and 3% (W/W).

Determination of pasting properties of the prepared flour blends

The pasting behavior of the prepared flour blends was tested using Viscoamylograph instrument (C.W. Brabender, South Hackensack, NJ) according to AACC (2000).

Processing of pasta

Pasta samples were processed according to Hallabo et al. (1985) using Pasta Matic 1000 Simac Machine Corporation, Milano, Italy. One hundred grams of flour were placed in the machine mixer and water (33%) was added then mixed under vacuum. The processed pasta samples were dried overnight at 75°C (Ehret GmbH, Germany). Dried pasta samples were stored in polyethylene bags until analysis.

Cooking quality of the pasta

Pasta samples (10 g) were cooked in 500 ml beakers using 1:15 ratio (W/V) of pasta to the water. The cooked weight (g) of pasta was recorded. Cooking loss and volume increase of cooked pasta were measured according to AACC (2000).

Color attributes of pasta

Color attributes (L*, a*, and b*) of pasta samples were determined using Hunter instrument (Hunter, Lab scan XE) with the CIE color scale. Total color differences (ΔE) were calculated as:

 $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5}$

Organoleptic properties of pasta

Organoleptic properties of cooked pasta samples including color, appearance, flavor,

tenderness, and stickiness were investigated by ten panelists according to Hallabo et al. (1985).

Microstructure of pasta

The internal microstructure of uncooked pasta samples was examined by a Jeol Scanning Electron Microscope type JXA 840A, Japan. Photomicrographs were taken at the magnification of 500X.

Statistical analysis

The obtained data were assessed by analysis of variance (ANOVA) and least significant difference (LSD) test at the level of significance (p < 0.05) (McClave & Benson, 1991).

Results and Discussion

Amino acid profiles of wheat proteins

The amino acid profiles of wheat proteins are presented in Table 1. Albumin fraction recorded the highest value of essential amino acids being 45.7 g/100 g sample and was rich in leucine (10.7 g/100 g sample), valine (8.1 g/100 g sample), and histidine (4.3 g/100 g sample), but it had the lowest threonine content (2.0 g/100 g sample) as compared to the other protein fractions. Globulin fraction was rich in lysine (12.2 g/100 g sample) and threonine (4.5 g/100 g sample), while it recorded lower values for other amino acids. Glutenin and gliadin fractions showed similar amino acid profiles. However, the gliadin fraction showed higher values of phenylalanine (5.6 g/100 g sample) and isoleucine (4.5 g/100 g sample), while the glutelin fraction had the highest value of methionine (1.7 g/100 g sample). Similar observations were noticed by Byers et al. (1983) and Shewry (2003).

Regarding phenylalanine content, the results showed that the storage protein (gliadin and glutelin) had about 88.8%, while globulin and albumin fractions had about 11.2% of the phenylalanine content. Moreover, data in Table 1 showed that gliadin fraction had about 46.3% of the total phenylalanine in wheat flour. Therefore, it was expected that de-gliadin wheat flour could be suitable for the production of low phenylalanine pasta.

Pasting properties of dough

Pasting properties of de-gliadin wheat flour and their blends with gum Arabic, pectin, or CMC at the levels of 1, 2 and 3% are shown in Table 2. Data in this Table showed that the viscoamylogram parameters of wheat flour dough were 63 and 85°C for transition temperature and temperature of maximum viscosity, and 370, 210 and 250 BU for, maximum, break down and setback viscosities, respectively compared to 54°C, 90°C, 2340 BU, 840 BU and 1680 BU for degliadin wheat flour.

TABLE 1. Amino acid profiles of wheat protein fractions (g/100 g sample)

Amino acids		Wheat J	oroteins	
Annuo acius	Albumin	Globulin	Gliadin	Glutelin
Essential Amino Acids (EAA)				
Methionine	0.5	0.4	1.5	1.7
Threonine	2.0	4.5	2.3	3.3
Isoleucine	4.1	1.4	4.5	3.9
Leucine	10.7	9.2	7.2	6.9
Valine	8.1	2.2	4.4	4.5
Phenylalanine	5.0	3.2	5.6	4.8
Histidine	4.3	2.2	2.3	2.4
Lysine	11.0	12.2	0.7	2.3
Total EAA	45.7	35.3	28.5	29.8
Non Essential Amino Acids (NEAA)				
Aspartic acid	7.9	6.3	3.0	3.5
Serine	4.7	9.1	4.8	5.3
Glutamic acid	6.5	4.5	37.0	31.1
Proline	6.3	3.2	13.1	10.0
Glycine	3.1	5.6	1.8	4.2
Alanine	5.6	4.3	2.3	3.1
cystine	6.7	12.6	2.7	2.5
Tyrosine	3.4	2.3	2.6	3.6
Arginine	7.5	14.5	2.7	4.2
Total NEAA	51.7	62.4	70.0	67.5
Total AA	97.4	97.7	98.5	97.3

Tryptophan was not determined.

Addition of pectin or gum arabic increased transition temperature (60-71°C) at all used concentrations. Results also showed that all tested hydrocolloids at all levels decreased the maximum viscosity (2300-1370 BU) compared with de-gliadin sample. The highest reduction was recorded for 3% pectin followed by 3% CMC. Similar results were reported for the cellulose gum solutions by Glicksman (1982). Setback value refers to the retrogradation behavior of starch paste during the cooling phase. Breakdown viscosity and setback viscosity showed also reduced values compared to de-gliadin wheat flour. It was found that the addition of pectin caused the highest breakdown and setback viscosity reduction. This could be attributed to the emulsifying ability of the tested hydrocolloids which rejected the development of hydrogen bonds between the hydroxyl groups on the amylose and amylopectin of starch granules in de-gliadin wheat flour.

Cooking quality of low phenylalanine pasta

The cooking quality parameters of pasta prepared from de-gliadin wheat flour and their blends with hydrocolloids are presented in Table 3. Data in this table showed that the cooking loss of the de-gliadin pasta (25.82%) was higher than that of the control pasta sample (10.6%). The higher cooking loss of de-gliadin pasta could be attributed to the absence of the gluten network as a result of the separation of the gliadin fraction. The Gluten network represents the main matrix that embeds the starch granules and restricts their leaching out during the cooking of pasta (Yaseen & Ahmed, 1999). It was found that the addition of hydrocolloids decreased the loss during cooking of pasta to 13.98, 14.77, and 13.25% in the case of 3% pectin, gum arabic, and CMC, respectively. Results also showed a difference in weight increase being 200% for the control sample and 77% for the de-gliadin pasta. The addition of hydrocolloids improved the weight increase to 81-86%.

Dick & Youngs (1988) reported that the ideal weight increase of cooked spaghetti is around 300%. Results also showed that the volume of control pasta increased by 350%, while it was 227% for de-gliadin pasta and 250-360% for pasta containing hydrocolloids. This means that the addition of hydrocolloids increased the volume and reduced the cooking loss. Such effect could be attributed to the swelling properties of hydrocolloids. Abcassis et al. (1989) reported that pasta could be manufactured from nongluten cereals such as maize or sorghum with some difficulties using special technologies. Furthermore, they mentioned that the absence of a gluten network in these cereals could be overcome either by corn flour pre-gelatinization process or by adding wheat flour to maize in order to provide an insufficient gluten network.

Sample	Transition temp. (°c)	Maximum viscosity (BU)	Temp. at maximum viscosity (°c)	Breakdown viscosity (BU)	Setback viscosity (BU)
Control	63	370	85	210	250
De-gliadin	54	2340	90	840	1680
Pectin					
1%	71	2000	95	390	1390
2%	66	1840	91	610	1570
3%	61.5	1370	89	460	1290
Gum Arabic					
1%	60	1800	87	800	1550
2%	63	1540	87	590	1230
3%	60	1500	87	600	1800
Carboxymethylcellul	ose				
1%	54	2300	88.5	720	1460
2%	54	1900	91.5	860	1320
3%	55.5	1480	90	760	1520

TABLE 2. Viscoamylogram parameters of wheat flour dough

BU = Brabender Unit

	Weight increase	Volume increase	Cooking loss
Sample	(%)	(%)	(%)
Control	200	350	10.6
De-gliadin	77.01	227	25.82
Pectin	//		
1%	81.46	250	19.15
2%	81.70	300	14.33
3%	86.17	325	13.98
Gum arabic			
1%	81.33	280	19.57
2%	81.42	285	19.39
3%	82.14	290	14.77
Carboxymethylcellulose			
1%	83.09	300	20.38
2%	83.57	350	14.18
3%	84.32	360	13.25

TABLE 3. Cooking quality of low phenylalanine pasta

The color quality of low phenylalanine pasta

The color parameters of low phenylalanine pasta are presented in Table 4. Regarding Hunter color values of uncooked pasta, de-gliadin pasta was darker in color as the lightness "L*» value was 54.13 compared to 70.02 for the control. Results showed that all tested hydrocolloids at all levels decreased the lightness which also increased with increasing the hydrocolloid concentration.

Results of "a*" values indicated that degliadin pasta had more redness value than the control. Pasta made from de-gliadin wheat flour with either pectin at 2 or 3% or gum arabic at 1% had more redness values than control. The addition of either pectin at 1% or gum arabic at 2 or 3% decreased the redness of pasta. The highest reduction was recorded with gum arabic at 2 or 3% where "a*" value decreased to 3.82 and 3.53 compared to 11.55 for de-gliadin pasta. Results also revealed that CMC had no clear effect on this factor.

Results in Table 4 also indicated that "b*" values (degree of yellowness) of pasta were 18.98 for the control sample and increased as a result of the separation of gliadin to 26.81. The addition of either pectin or CMC at all levels increased pasta yellowness. Maximum increase (36.21) was recorded when pectin was added at 2%. The addition of gum arabic at 2 or 3% decreased the yellowness of pasta.

The differences in color « \square E» between pasta prepared from de-gliadin wheat flour and those containing hydrocolloids increased as the level of hydrocolloids increased. CMC had the lowest values of « Δ E» (4.60 – 11.09), while gum arabic had the highest values (11.37- 40.42). This indicated that pasta containing hydrocolloids was darker in color as the lightness values decreased and the redness values increased.

Organoleptic properties of low phenylalanine pasta

The organoleptic properties of the produced pasta samples including appearance, flavor, color, stickiness and tenderness were evaluated. Results Table 5 showed significant differences (at 0.05) between control and de-gliadin pasta and those made from de-gliadin wheat flour with hydrocolloids. Significant differences were also found between pasta made using different types of hydrocolloids and within the same type at different levels of addition. De-gliadin pasta was rated lower than the control and those containing hydrocolloids. The addition of CMC at 3% improved all tested attributes as there were no significant differences between pasta made using CMC at 3% and control pasta. Pectin at the same level also gave a good effect on the tested attributes. These results agree with those previously reported by Yaseen & Ahmed (1999).

Amino acid profiles of low phenylalanine pasta

Data presented in Table 6 showed the amino acids composition and protein content of low phenylalanine pasta samples. Gliadin separation decreased the protein content of de-gliadin pasta by 39.1%. As a consequence, the total amino acid contents of all processed pasta samples were decreased compared to the control (9.4 g/100 g dry sample). The total amino acids content of de-gliadin pasta were reduced by 39.2% compared to control pasta. Essential and nonessential amino acid contents showed the same trend. De-gliadin pasta samples showed high glutamic, proline, arginine and tyrosine contents, and low methionine, lysine and cystine contents. Moreover, all samples had lower phenylalanine content than the control one. The reduction in phenylalanine reached 42.6% in de-gliadin pasta (Fig. 1).

	Lightness	Redness	Yellowness	Color differences
Sample	(L*)	(a*)	(b*)	(ΔE)
Control	70.02	2.95	18.98	0.00
De-gliadin	54.13	11.55	25.81	0.00
Pectin				
1%	52.86	10.43	27.76	2.58
2%	48.16	13.06	36.21	2.38
3%	37.60	12.75	33.22	18.15
Gum arabic				
1%	48.88	14.53	35.44	11.37
2%	39.25	3.82	5.94	26.00
3%	19.96	3.53	5.77	40.42
Carboxymethylcellulo	se			
1%	51.08	9.78	28.77	4.60
2%	47.47	11.99	22.92	4.00
3%	44.15	11.54	30.65	11.09

TABLE 4. Color attributes of low phenylalanine pasta

TABLE 5. Organoleptic properties of low phenylalanine pasta

Come la	Appearance	Color	Flavor	Tenderness	Stickiness
Sample	(10)	(10)	(10)	(10)	(10)
Control	9.50 ª	9.38 ª	9.20 ª	8.85 ª	8.66 ^a
De-gliadin	5.83 ^d	4.83 ^d	5.33 ^d	3.83 ^g	3.83 ^f
Pectin					
1%	6.33 ^{cd}	6.83 ^{bc}	6.50 ^{cd}	4.33 ^{fg}	4.83 ^{cd}
2%	6.00 ^d	7.17 ^b	7.00 ^{bcd}	6.50 ^{bcd}	8.00 ^{abc}
3%	8.00 ^{ab}	7.83 ^b	8.00 ^{ab}	7.67 ^{ab}	8.17 ^{abc}
Gum arabic					
1%	7.17 ^{bcd}	5.50 ^{cd}	6.50 ^{cd}	4.83 ^{efg}	5.33 ^{ef}
2%	7.50 ^{bc}	6.50 ^{bc}	6.50 ^{cd}	5.83 ^{def}	6.17 ^{de}
3%	7.50 ^{bc}	6.67 ^{bc}	6.50 ^{cd}	7.17 ^{bcd}	8.00 ^{abc}
Carboxymethylc	ellulose				
1%	7.50 ^{bc}	7.33 ^b	7.17 ^{bcd}	6.00 ^{cde}	7.00^{bcd}
2%	7.67 ^{bc}	7.67 ^b	7.50 ^{bc}	7.50 ^{abc}	8.33 ^{ab}
3%	9.33ª	9.33ª	9.17ª	8.83ª	8.67ª
LSD	1.854	1.46	1.447	1.568	1.484

Means within the same column designed by the same letter are not significantly different

Amino acid	i	;		Pectin			Gum arabic		Carbo	xymethylce .	llulose
	Control	De-gliadin	1%	2%	3%	1%	2%	3%	1%	2%	3%
Essential Amino Acids (1	EAA)										
Valine	0.41	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Threonine	0.18	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Methionine	0.23	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Isoleucine	0.92	0.56	0.55	0.55	0.55	0.55	0.55	0.56	0.55	0.55	0.55
Leucine	0.28	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Phenylalanine	0.54	0.31	0.32	0.33	0.30	0.33	0.31	0.33	0.33	0.33	0.30
Histidine	0.34	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Lysine	0.41	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.24
Total EAA	3.31	2.00	2.00	2.00	1.93	2.01	1.98	2.02	2.01	2.00	1.97
Non-Essential Amino Ac	ids (NEAA)										
Glutamic acid	2.98	1.82	1.81	1.81	1.80	1.81	1.81	1.82	1.81	1.81	1.80
Aspartic acid	0.48	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Serine	0.30	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Proline	0.95	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.57
Glycine	0.31	0.19	0.18	0.18	0.18	0.18	0.18	0.19	0.18	0.18	0.18
Alanine	0.33	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
cystine	0.10	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Tyrosine	0.25	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Arginine	0.39	0.24	0.24	0.24	0.23	0.24	0.24	0.24	0.24	0.24	0.23
Total NEAA	6.08	3.70	3.68	3.67	3.67	3.68	3.69	3.70	3.69	3.67	3.66
Total AA	9.40	5.70	5.68	5.67	5.64	5.69	5.68	5.72	5.70	5.67	5.63
Protein	10.55	6.43	6.39	6.38	6.37	6.39	6.40	6.42	6.40	6.38	6.36

Results in Table 6 also showed that about 200 g intake of the processed low phenylalanine pasta could represent the daily phenylalanine requirement of the phenylketonuria patients. Such amount of pasta (200 g) contained about 500 mg phenylalanine and could be considered as the maximum value intake. These findings are almost similar to those reported by Courtney-Martin et al. (2002) and Walter & White (2004).

Microstructure of pasta

The microstructure of the processed low phenylalanine pasta was investigated using the Scanning Electron Microscopy technique (Fig. 2). The control pasta sample showed a smooth matrix from the continuous protein layers that envelope the air bubbles. Starch particles in the control pasta were characterized with distinctive spherical and lenticular shapes with small diameters, which were embedded in the gluten network that was observed on the surface of the granules.

The photomicrographs of de-gliadin pasta samples showed that the separation of gliadin from wheat flour affected its internal structure where the air cell disappeared and a discontinuous gluten matrix appeared. Starch particles were characterized with larger size and heterogeneous structure. Moreover, the rod of spaghetti pasta showed compacted areas rather than the latticed structure of the cells. These results indicate that separation of gliadin gave weak, fragile, and breakable pasta products. Yaseen & Ahmed (1999) reported that spaghetti containing starch granules differed due to their less compact structure. More large starch granules and less small ones were interspaced. This structure was predominant in spaghetti containing a high level of starch substitution. These observations explain the cooking quality results and confirm the viscoamylograph results.



Fig.1. Phenylalanine content of pasta



Fig. 2. Photomicrostructure of pasta

Conclusion

In conclusion, the above-mentioned results indicated that hydrocolloids i.e. gum Arabic, pectin, and CMC could be separately added to de-gliadin wheat flour to improve the quality parameters of pasta including dough handling properties, cooking quality (weight increase, volume increase, and cooking loss), and microstructure. Under processing conditions, acceptable pasta could be produced using degliadin wheat flour containing 2% pectin or 3% CMC. Such pasta products contained low phenylalanine content and could be used for PKU patients.

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