

2 **Production of functional pasta with low phenylalanine based on potato and**  
3 **tapioca starches**

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5 **Short Title: Pasta for phenylketonuria patients**

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11  
12 **ABSTRACT**

13 Phenylketonuria (PKU) is a genetic disorder necessitating a low-protein and phenylalanine diet.  
14 This study aimed to explore the feasibility of producing a low-protein pasta using potato and  
15 tapioca starches. The pasta formulation substituted semolina flour with a blend of potato and  
16 tapioca starches. Date kernel fiber and xanthan gum were incorporated as prebiotic compounds  
17 and texture enhancers, respectively. Physicochemical (moisture, fat, total ash, protein,  
18 phenylalanine, cooking loss, cooking time, color indexes, and hardness) and sensory properties  
19 (texture, flavor, color, and overall acceptability) were evaluated and compared against the control  
20 sample (based on semolina flour). The results demonstrated no significant alteration in moisture  
21 and fat content upon substitution, but a significant decrease in ash and protein content ( $p < 0.05$ ).  
22 Consequently, phenylalanine levels decreased from 530.58 mg/100 g in the control sample to  
23 24.49-26.60 mg/100 g in the pasta. Replacing flour with starches increased cooking loss, reduced  
24 cooking time, and diminished pasta hardness compared to the control ( $p < 0.05$ ). The pasta exhibited  
25 higher  $L^*$  and lower  $a^*$  and  $b^*$  values than the control. Sensory evaluation revealed that the pasta  
26 containing 35% potato starch and 40% tapioca starch attained the highest scores, indicating its  
27 favorable acceptability. Overall, this study suggests that the combination of potato and tapioca  
28 starches, along with date kernel fiber and xanthan gum, enables the production of and low-protein  
29 pasta suitable for PKU patients.

30 **Keywords:** Date kernel fiber, Low-protein pasta, Phenylketonuria, Potato starch, Tapioca starch.

31 **1. INTRODUCTION**

32 Pasta is one of the most popular and common staple foods in the human diet, with convenient  
33 consumption, favorable organoleptic characteristics and high nutritional value. Pasta contains

34 digestible carbohydrate and has little fat. In addition, pasta has low price and its shelf life is long.  
35 The main ingredients of pasta formulation are durum wheat flour (semolina) and water (Bresciani,  
36 Pagani et al. 2022).

37 Phenylketonuria (PKU) is a genetic disorder caused by the deficiency of the phenylalanine  
38 hydroxylase enzyme. This enzyme causes the conversion of phenylalanine to tyrosine, and the  
39 lack of this enzyme causes an increase in the accumulation of phenylalanine in the blood and brain  
40 (MacDonald, Van Wegberg et al. 2020). PKU can lead to severe neurological problems such as  
41 irreversible mental disability if not controlled. The complications of this disease can be prevented  
42 by severely limiting protein-containing foods and consuming food products with low  
43 phenylalanine content (McWhorter, Ndugga-Kabuye et al. 2022).

44 Pasta cooking quality is influenced by protein content, with gluten being crucial for elasticity and  
45 chewability. Gluten-free pastas can be made using an appropriate ratio of proteins, hydrocolloids,  
46 and water. Research focuses on developing gluten-free products using non-wheat flours, dairy  
47 products, and emulsifiers to improve structure, mouth feel, acceptability, and shelf-life. However,  
48 issues include the absence of gluten and achieving acceptable quality without traditional wheat  
49 ingredients (Ungureanu-Iuga, Dimian et al. 2020, Gasparre and Rosell 2023). To develop gluten-  
50 free pasta, polymer ingredients such as starches and hydrocolloids are needed to imitate the  
51 viscoelastic behavior of gluten in the product batter and create a favorable texture, mouth feel and  
52 acceptability (Zoghi, Mirmahdi et al. 2021, Zhang, Zhang et al. 2022). Xanthan gum is one of the  
53 most widely used hydrocolloids for the development of gluten-free or gluten-reduced products  
54 (Palavecino, Bustos et al. 2017). Potato (*Solanum tuberosum* L.) is an excellent source of  
55 carbohydrates and its starch content is about 75-80% based on dry weight (Dupuis and Liu 2019).  
56 The unique features of potato starch compared to cereal starches include the longer chain length  
57 of amylopectin and amylose, greater granular size and purity, ability to exchange specific cations  
58 with an effect on viscosity behavior, presence of phosphate ester groups on amylopectin, and the  
59 formation of a strong viscoelastic gel. These unique features determine the sensory attributes and  
60 quality of gluten-free pastas (Raj, Dalal et al. 2020). Tapioca or cassava starch is a gluten-free  
61 product that has favorable characteristics for application in the food industry. This starch has a  
62 high availability and low price, and it controls or changes the characteristics of foods such as  
63 appearance, texture, consistency, and storage stability (Reddy, Kimi et al. 2016).

64 The substitution of wheat flour with starch in gluten-free products can reduce fiber and nutrient  
65 intake. To enhance the nutritional value of these products, various sources of dietary fiber and  
66 nutritional compounds are used, with fruits and vegetables being crucial sources of dietary fiber  
67 (Zhang, Dongye et al. 2023). Dietary fibers are among the prebiotic compounds, and are  
68 indigestible and have various health benefits, especially the regulation of human intestinal activity.  
69 So, recently attention has been directed towards the development of prebiotic products (Abedinia,  
70 Alimohammadi et al. 2021). Date fruit belongs to *Arecaceae* family, and is native to tropical and  
71 subtropical regions (Hussain, Farooq et al. 2020). Date consist of pulp and kernel, and the kernels  
72 make up 5% to 15% of the total weight of fruit. Date kernel is the most important waste of date  
73 processing factories, and is rich in fat, protein and dietary fiber (Alharbi, Raman et al. 2021). The  
74 functional characteristics of date kernels have been found to consist of 22.5–80.2% dietary fibre,  
75 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, and 0.9–1.8% ash. Due to their high dietary  
76 fibre content, date seeds have a significant nutritional value and may be used to manufacture dishes  
77 high in fibre and as dietary supplements. Date seeds have a high dietary fibre content since they  
78 are generated in huge quantities as a waste product (Samea and Zidan 2019).

79 In this research, for the production of functional pasta with reduced protein and phenylalanine,  
80 wheat flour was replaced with different levels of potato (PS) and tapioca starches (TS), and xanthan  
81 gum and date kernel fiber (DKF) were used as texture improver and prebiotic agent, respectively,  
82 and the effect of starches on the physicochemical properties, cooking quality, sensory  
83 characteristics and protein and phenylalanine content of the produced pastas were evaluated.

84

## 85 **2. MATERIALS AND METHODS**

### 86 **2.1. MATERIALS**

87 Semolina flour was prepared from Zar Flour of Karaj (Iran). Potato and tapioca starches were  
88 also purchased from Stage Company (Iran). Xanthan gum,  $\beta$ -carotene and date kernel fiber were  
89 purchased from Provisco Company (Germany), Merck Company (Germany) and Flavinea  
90 Company (Iran), respectively. The chemical composition of semolina flour, PS, TS and DKF used  
91 in this research was determined using the AACC standard method (AACC 2000) and their values  
92 are listed in Table 2. All the chemicals used for the tests were also purchased from Merck  
93 (Germany).

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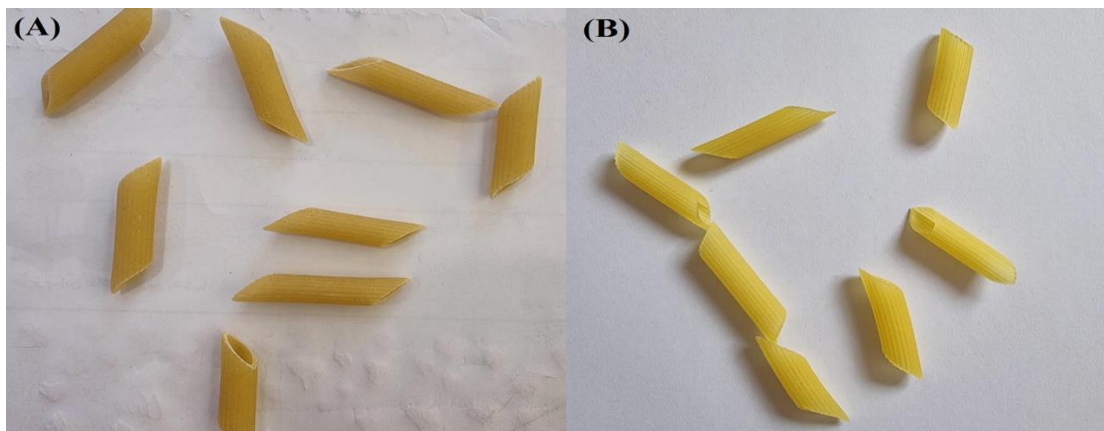
## 96 2.2. Preparation of pasta treatments

97 The formulation of the control pasta (*penne*) was as follows: 76% semolina flour, 1% DKF,  
98 0.015%  $\beta$ -carotene, and 23.985% water. In the formulation of low-protein pastas, the combination  
99 of different levels of PS and TS (Table 1) completely replaced semolina flour. Xanthan gum (1%)  
100 was used as a texture improver. To prepare pasta treatments, dry ingredients were first mixed  
101 together in a mixer (Anselmo, Italy) for 3 min.  $\beta$ -carotene was dissolved in the formulation water  
102 and gradually added to the dry ingredients and stirred for 20 min. The resulting mixture was  
103 extruded (La Monferrina Model P6, Roma, Italy) has an 8 mm x 3.4 mm cylinder-shaped diameter  
104 at 45°C and under pressure of 0.6 mm Hg. During the extrusion process, the temperature of the  
105 batter taken out of the mold was subjected to a flow of water at 20°C so that the pasta doesn't stick  
106 together and doesn't losses its shape. The produced pastas were collected in a basket and placed  
107 in a dryer for 24 h at a variable range of 20-80°C until they were completely dry and their moisture  
108 content was less than 12%. After cooling, the produced pastas were packed in polypropylene bags  
109 and kept at 18°C. Figure 1 shows the samples prepared for this study.

110 **Table 1.** The formulations of pasta treatments (g/100 g dry basis) with constant amounts of  $\beta$ -  
111 Carotene, DKF, semolina flour (0.015, 1, and 75%, respectively) and water (in the amount to form  
112 a dough).

Treatments	PS	TS	Xanthan gum
T0 (control)	-	-	-
T1	75	-	1
T2	65	10	1
T3	55	20	1
T4	45	30	1
T5	35	40	1
T6	25	50	1
T7	15	60	1
T8	-	75	1

113 DKF: date kernel fiber, PS: potato starch, TS: tapioca starch.



114 **Figure 1.** Pasta treatments (A) control; (B) pasta containing 35% PS+40% TS.  
115

## 116 **2.3. Analysis of physicochemical properties of pasta**

### 117 2.3.1. Proximate and physicochemical analysis

118 Moisture, fat, total ash and protein content of pastas were determined using the AOAC standard  
119 method (44-16, 30-01, 44-15, and 12-46, respectively) (AACC 2000). The cooking loss values of  
120 the **pasta samples were** measured using the Iranian national standard method number 213 **by**  
121 **dividing pastas weight after by before of cooking**. The color analysis of the **raw pastas** was done  
122 through three indexes of L\* (bright), a\* (red/green) and b\* (yellow/blue) and using a colorimeter  
123 (Hunterlab, America) (Milde, Chigal et al. 2020). The hardness of the cooked pastas was **measured**  
124 using a Brookfield CT3 texture analyzer (England) with a cylindrical probe (35 mm) and the test  
125 of single compression. Compression up to 80% of the initial thickness of the pasta was done by  
126 the device probe at a speed of 1 mm/s, and the force required for this work was reported as N  
127 (Piwińska, Wyrwisz et al. 2016).

### 128 129 2.3.2. Measurement of phenylalanine

130 To measure the phenylalanine content of raw pastas, an HPLC device (Waters, America)  
131 equipped with a UV detector and RP-C18 column with particle size of 5  $\mu\text{m}$ , and dimensions of  
132 150 mm  $\times$  39 mm was used. Initially, the sample (0.5 g) was mixed with 6 M HCl (8 mL) and after  
133 airing, the resulting mixture was hydrolyzed in an oven for 22 h at a temperature of 110  $^{\circ}\text{C}$ . The  
134 obtained hydrolyzates were first centrifuged at 5000 rpm for 10 min, neutralized by NaOH 3M  
135 and then injected into HPLC device in a volume of 20  $\mu\text{L}$ . The mobile phase consisted of a mixture  
136 of acetonitrile and phosphate buffer (pH = 3.5) in a ratio of 98 to 2, and its flow rate was set at 0.8  
137 mL/min (Yaseen and Shouk 2011).

## 138 139 **2.4. Sensory evaluation of pasta**

140 The sensory evaluation of the cooked pasta was done using 10 trained panelists and according to  
141 the 7-point Hedonic test (7= excellent sample and 1=very bad sample), and the panelists rated the  
142 texture, flavor, color and overall acceptability of the pasta treatments. Preparation of pastas for  
143 sensory evaluation was done by heating sample (50 g) in boiling water (250 mL) with 2% salt  
144 **according to Table 4, for each sample separately and was evaluated by the panelists** (Shogren,  
145 Hareland et al. 2006).

146  
147

## 148 2.5. Statistical analysis of data

149 The results of the experiments were analyzed to investigate the significant difference between  
150 the data through one-way analysis of variance using SPSS 22.0 software. Duncan's multi-range  
151 test was used to compare the mean of the treatments at the 95% probability level.

## 152 3. RESULTS AND DISCUSSION

### 154 3.1. Chemical composition of pasta

155 The results of examining the chemical composition of pasta (**Table 3**) showed that the amounts  
156 of moisture, ash, fat and protein of pasta treatments were in the range of 10.31-10.83%, 0.61-  
157 0.72%, 1.10-1.14%, and 0.94-11.13%, respectively. Substitution of semolina flour in pasta  
158 formulation with the combination of starches, DKF and xanthan gum didn't have a significant  
159 effect on the moisture and fat content of produced pastas, but significantly reduced the amounts of  
160 ash and protein ( $p < 0.05$ ). The **decrease** in the ash content of pasta is probably because the amount  
161 of total ash in PS (0.23%) and TS (0.12%) is less than that of semolina flour (0.74%). The lower  
162 protein content of PS (0.17%), TS (0.14%) and DKF (2.23%) compared to semolina flour (13.62%)  
163 is also the reason for reducing the protein content of pasta with flour replacement. Yaseen and  
164 Shouk (Yaseen and Shouk 2011) also reported that due to the lower **ash**, fat and protein content of  
165 corn starch compared to semolina flour, increasing the replacement of flour caused a significant  
166 decrease in ash, fat and protein content of the pasta. Similarly with these results, Mossadegh et al.  
167 (Mossadegh, Tavakoli et al. 2018) and Crizel et al. (Crizel, Rios et al. 2015) observed that the  
168 addition of potato fibre and orange fibre to the pasta formulation didn't significantly change the  
169 fat content of the samples, however reduced the protein content.

170

171 **Table 2.** The chemical composition of semolina flour, PS, TS and DKF used in this research.

Composition	Semolina flour	PS	TS	DKF
Moisture (%)	11.83 ± 0.37 <sup>c</sup>	12.65 ± 0.19 <sup>b</sup>	13.93 ± 0.22 <sup>a</sup>	6.92 ± 0.16 <sup>d</sup>
Fat (%)	1.16 ± 0.03 <sup>c</sup>	1.28 ± 0.01 <sup>b</sup>	0.41 ± 0.03 <sup>d</sup>	3.14 ± 0.05 <sup>a</sup>
Protein (%)	13.62 ± 0.09 <sup>a</sup>	0.14 ± 0.02 <sup>c</sup>	0.17 ± 0.04 <sup>c</sup>	2.23 ± 0.05 <sup>b</sup>
Carbohydrate (%)	57.80 ± 0.41 <sup>d</sup>	85.52 ± 0.47 <sup>a</sup>	83.44 ± 0.50 <sup>b</sup>	79.94 ± 0.38 <sup>c</sup>
Total ash (%)	0.74 ± 0.03 <sup>b</sup>	0.12 ± 0.02 <sup>d</sup>	0.23 ± 0.02 <sup>c</sup>	1.02 ± 0.05 <sup>a</sup>
Fiber (%)	3.46 ± 0.27 <sup>b</sup>	0.61 ± 0.08 <sup>c</sup>	0.36 ± 0.11 <sup>d</sup>	73.94 ± 0.46 <sup>a</sup>

172 Values represent mean (n=3) ± SD. Different letters in each row represent significant difference at 5% level of  
173 probability among samples. DKF: date kernel fiber, PS: potato starch, TS: tapioca starch.

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**Table 3.** Chemical composition of pasta treatments.

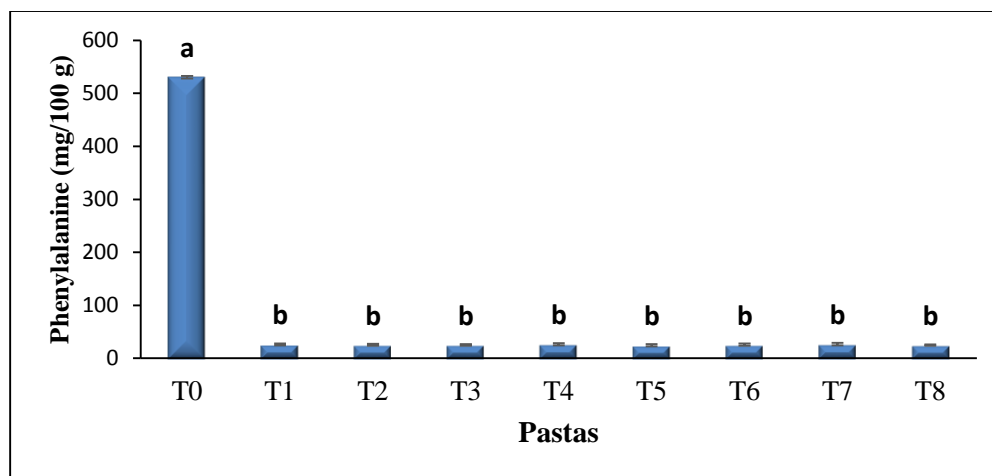
Treatments	Moisture (%)	Fat (%)	Total ash (%)	Protein (%)
T0 (control)	10.31 ± 0.37 <sup>a</sup>	1.10 ± 0.05 <sup>a</sup>	0.72 ± 0.01 <sup>a</sup>	11.13 ± 0.04 <sup>a</sup>
T1	10.83 ± 0.24 <sup>a</sup>	1.13 ± 0.03 <sup>a</sup>	0.64 ± 0.01 <sup>b</sup>	0.95 ± 0.07 <sup>b</sup>
T2	10.82 ± 0.30 <sup>a</sup>	1.12 ± 0.03 <sup>a</sup>	0.66 ± 0.02 <sup>b</sup>	1.01 ± 0.02 <sup>b</sup>
T3	10.81 ± 0.27 <sup>a</sup>	1.10 ± 0.04 <sup>a</sup>	0.62 ± 0.02 <sup>b</sup>	0.97 ± 0.05 <sup>b</sup>
T4	10.74 ± 0.19 <sup>a</sup>	1.14 ± 0.01 <sup>a</sup>	0.64 ± 0.03 <sup>b</sup>	1.00 ± 0.04 <sup>b</sup>
T5	10.62 ± 0.35 <sup>a</sup>	1.13 ± 0.03 <sup>a</sup>	0.64 ± 0.02 <sup>b</sup>	0.95 ± 0.06 <sup>b</sup>
T6	10.63 ± 0.14 <sup>a</sup>	1.13 ± 0.02 <sup>a</sup>	0.61 ± 0.04 <sup>b</sup>	0.94 ± 0.07 <sup>b</sup>
T7	10.60 ± 0.21 <sup>a</sup>	1.14 ± 0.02 <sup>a</sup>	0.63 ± 0.02 <sup>b</sup>	1.04 ± 0.08 <sup>b</sup>
T8	10.68 ± 0.28 <sup>a</sup>	1.12 ± 0.04 <sup>a</sup>	0.64 ± 0.01 <sup>b</sup>	0.99 ± 0.05 <sup>b</sup>

179 Values represent mean (n=3) ± SD. Different letters in each column represent significant difference at 5% level of  
 180 probability among samples.

181

### 182 3.2. Phenylalanine content of pasta

183 PKU is a genetic disease caused by the deficiency of the phenylalanine hydroxylase enzyme,  
 184 which converts phenylalanine to tyrosine. This disease causes severe neurological complications  
 185 including irreversible mental disability (MacDonald, Van Wegberg et al. 2020). Therefore, the  
 186 level of protein and phenylalanine in the food of these patients is very vital and important. The  
 187 results of the **examination** the phenylalanine content of pasta (Figure 2) showed that the pasta  
 188 based on semolina flour (control) had the highest level of phenylalanine (530.58 mg/100 g **dry**  
 189 **matter**), and the complete replacement of wheat flour in the pasta formulation with different levels  
 190 of PS and TS caused a significant decrease in the amount of phenylalanine in the produced low-  
 191 protein pastas (p<0.05). However, there was no significant difference between the phenylalanine  
 192 content of different pasta treatments containing starches, and the phenylalanine content of these  
 193 treatments were in the range of 24.49-26.60 mg/100 g **dry matter**. The lower phenylalanine content  
 194 of the pasta compared to the control sample is due to the much lower protein content of PS and TS  
 195 compared to semolina flour. In this regard, Özboy (2002) stated that the starches contain very low  
 196 amounts of protein and phenylalanine and are therefore suitable for developing bakery products  
 197 for PKU patients. Yaseen et al. (Yaseen, Abd-El-Hafeez et al. 2011) reported that with the increase  
 198 of corn starch level in Egyptian bread formulation, the phenylalanine content of the samples  
 199 significantly decreased. Aghadadashzadeh Eslahi & Shams (Aghadadashzadeh Eslahi and Shams  
 200 2019) also showed in agreement with the results of this research that with the increase in the  
 201 replacement level of modified corn and tapioca starch in toast breads, their phenylalanine content  
 202 decreased significantly.



203  
 204 **Figure 2.** Phenylalanine content (mg/100 g) of pastas. Bars represent mean (n=3) ± SD. Different  
 205 letters on the bars indicate significant difference at 5% level of probability among samples.  
 206

### 207 3.3. Cooking loss of pastas

208 The cooking process is a key process in determining the desired quality of pasta. During the  
 209 cooking process of pasta and as a result of the application of heat and water absorption, various  
 210 reactions occur, including starch gelatinization, protein swelling, and increase in volume and  
 211 weight of pasta. Cooking loss indicates the exit of solid materials during the pasta cooking process  
 212 and is a main indicator that is widely used to show the overall performance of the cooking, and  
 213 indicates the resistance to decomposition during pasta cooking (Larrosa, Lorenzo et al. 2016).  
 214 During cooking, less solids are extracted from the pasta, the higher the quality of the cooked pasta  
 215 (Del Nobile, Baiano et al. 2005). The cooking loss values of the pastas were measured and the  
 216 results are given in Table 4. The pasta based on semolina flour (control) had the lowest amount of  
 217 cooking loss, and the replacement of flour with the combination of PS and TS caused a significant  
 218 increase in the cooking loss percentage of the low-protein and pastas (p<0.05). However, there  
 219 was no statistically significant difference between the cooking loss values of the pastas containing  
 220 starches, and the cooking loss values of these treatments were in the range of 8.10-8.59%. The  
 221 research has shown that flours with low protein and gluten-free have higher water absorption and  
 222 more cooking loss due to the absence of gluten network, because gluten reduces the absorption of  
 223 water by starch granules and prevents their leakage by preserving various compounds such as  
 224 gelatinized starches, carbohydrates and proteins (Yahyavi, Kamali Roustae et al. 2020). Hosseini  
 225 and Ardestani (Hosseini and Ardestani 2015) found protein reduction to be related to increase  
 226 cooking loss of pasta with protein removal. Overall, in gluten-free pasta, starch polymers are



227 effectively trapped in the matrix due to the absence of gluten, and the cooking loss of these  
228 products is higher compared to conventional pasta (Marti and Pagani 2013, Cui, Zhao et al. 2020).  
229 The maximum acceptable limit for pasta cooking loss is 10%, and cooking loss values less than  
230 10% indicate good pasta quality (Bouasla, Wójtowicz et al. 2017). Since the cooking loss values  
231 of all pastas produced in this research were less than 10%, so these treatments had good quality.  
232 Hydrocolloids such as xanthan gum help create a stronger network in gluten-free products, which  
233 traps starch granules and reduces cooking loss (Milde, Chigal et al. 2020). In the present study,  
234 due to the use of xanthan gum in the pasta formulations as a texture improver, **part** of the increased  
235 cooking loss due to the replacement of semolina flour with starches was compensated. Yaseen and  
236 Shouk (Yaseen and Shouk 2011) reported that the cooking loss of control pasta was 10.6%, and  
237 with the increase in the replacement of wheat flour with corn starch in the **pastas**, the cooking loss  
238 increased and at the level of 70% replacement, the cooking loss reached 22.0%. Tao et al. (Tao,  
239 Wang et al. 2020) also found that with the increase in the level of potato starch from 5% to 30%  
240 in the noodle formulation, the cooking loss of the noodles increased.

241

#### 242 **3.4. Cooking time of pastas**

243 During the cooking process of pasta in water, water absorption and starch granules gelatinization  
244 occurs. As a result of applying heat, the gluten is denatured at the same time as starch is gelatinized.  
245 However, gluten still **retains** a part of its water absorption ability (Palavecino, Bustos et al. 2017).  
246 The best and most appropriate cooking time is when a large number of starch granules have  
247 become gelatinized. The presence of gluten increases the cooking time of pastas by affecting the  
248 temperature of starch gelatinization (Hosseini and Ardestani 2015). Cooking time is generally a  
249 test that indicates the time required to cook pastas in boiling water and ready to eat (Milde, Chigal  
250 et al. 2020). The results of examining the cooking time of pasta (Table 4) showed that pasta based  
251 on semolina flour (control) had the longest cooking time (10.21 min), and the replacement of flour  
252 with the combination of PS and TS led to a significant reduction in the cooking time of low-protein  
253 pastas ( $p < 0.05$ ). By increasing the percentage of PS in the formulation, a decrease in the cooking  
254 time of the pasta was observed ( $p < 0.05$ ). The lowest cooking time was for the treatment containing  
255 75% PS (7.93 min), but there was no significant difference between this treatment and the pasta  
256 containing 65% PS+10% TS (8.04 min). The most effective factor in the effect of starches on the  
257 pasta cooking time is the pasting temperature, and starches with a higher pasting temperature have

258 a higher resistance to the swelling and breaking of starch granules and increase the cooking time  
 259 (Sonia et al., 2019). The research has shown that the pasting temperature of PS (67.9 °C) is lower  
 260 than that of TS (71.6 °C) (Park, Kim et al. 2009, Sharma, Oberoi et al. 2009). Therefore, with the  
 261 increase of PS in pasta formulation, a greater decrease in the cooking time of the produced pastas  
 262 was observed. Adding hydrocolloids can increase the cooking time of pasta because hydrocolloids  
 263 limit the moisture content of the product and delay the swelling of starch granules (Kaur, Singh et  
 264 al. 2002). Sonia et al. (Sonia, Julianti et al. 2019) observed that adding xanthan gum increased the  
 265 cooking time of gluten-free pasta. Chillo et al. (Chillo, Laverse et al. 2007) reported that the  
 266 addition of pre-gelatinized corn starch and CMC to spaghetti reduced the cooking time by 3.5  
 267 times in samples based on amaranth flour compared to samples based on semolina flour. In this  
 268 research, the reduction of cooking time was attributed to the absence of gluten in **pastas** without  
 269 wheat flour. In fact, it seems that the absence of the gluten network facilitates the diffusion of  
 270 water through the product matrix and reduces the time required for water to reach the center of the  
 271 product during the cooking process.

272 **Table 4.** Cooking loss and cooking time of pasta treatments.

Treatments	Cooking loss (%)	Cooking time (min)
T0 (control)	6.78 ± 0.40 <sup>b</sup>	10.21 ± 0.14 <sup>a</sup>
T1	8.59 ± 0.29 <sup>a</sup>	7.93 ± 0.09 <sup>f</sup>
T2	8.47 ± 0.38 <sup>a</sup>	8.04 ± 0.13 <sup>ef</sup>
T3	8.41 ± 0.26 <sup>a</sup>	8.25 ± 0.17 <sup>de</sup>
T4	8.23 ± 0.41 <sup>a</sup>	8.29 ± 0.10 <sup>d</sup>
T5	8.10 ± 0.24 <sup>a</sup>	8.35 ± 0.14 <sup>cd</sup>
T6	8.19 ± 0.31 <sup>a</sup>	8.40 ± 0.16 <sup>cd</sup>
T7	8.29 ± 0.46 <sup>a</sup>	8.56 ± 0.10 <sup>bc</sup>
T8	8.24 ± 0.33 <sup>a</sup>	8.70 ± 0.07 <sup>b</sup>

273 Values represent mean (n=3) ± SD. Different letters in each column represent significant difference at 5% level of  
 274 probability among samples.

### 275 3.5. Color of pasta

276  
 277 The color of pasta is one of the important parameters in product quality evaluation. Color is a  
 278 quality factor that affects consumer acceptance and product selection in the sale market (Xiong,  
 279 Chen et al. 2022). The color of **raw** pastas was determined using Hunterlab device and the results  
 280 are presented in Table 5. The control pasta had the lowest L\* (68.38) and the highest a\* (3.56) and  
 281 b\* (25.82), the color of low-protein and pasta containing PS and TS was brighter than the control  
 282 sample and less **red and yellow**. However, there was no statistically significant difference between  
 283 the color indexes of **pastas** containing starches, and the L\*, a\* and b\* values of these pasta **samples**  
 284 were in the range of 73.71-74.49, 0.76-0.86, and 20.30-20.79, respectively. The white color of PS

285 and TS is the reason for the increase in brightness of the color of gluten-free pastas compared to  
 286 the control. DKF also has a relatively white color. The researchers suggested that the ideal values  
 287 for the brightness index of the pasta color are greater than 60, and values less than 50 indicate the  
 288 overall darkness of the pasta (Luo, Wang et al. 2020). In agreement with the results of the present  
 289 study, Yaseen and Shouk (2011) also reported the lightening of the color of pasta with different  
 290 levels of corn starch compared to the control, and in their research, the intensity of redness and  
 291 yellowness of pasta containing corn starch was less than the control. The effect of xanthan gum in  
 292 brightening and yellowing the color of gluten-free pasta was also observed in the research of Milde,  
 293 Chigal et al. (2020).

294 **Table 5.** Color indexes of **raw pasta** treatments.

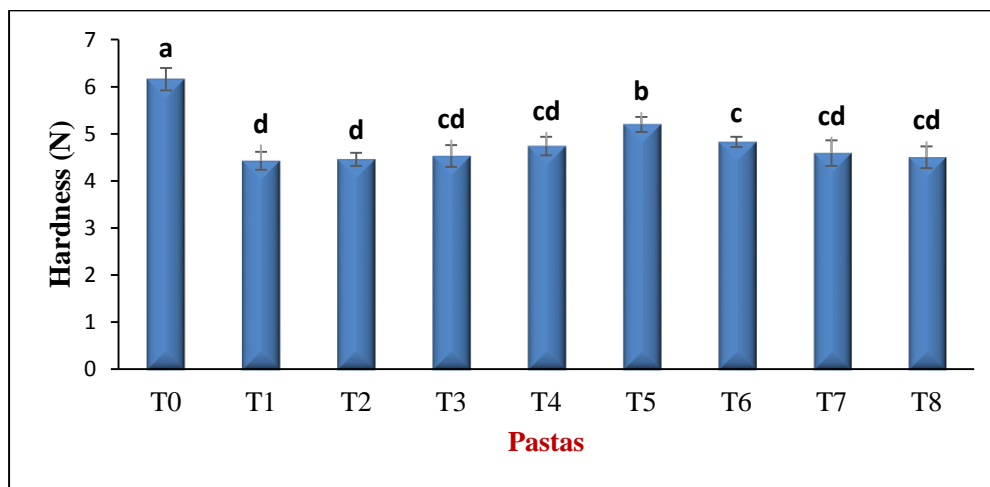
Treatments	L*	a*	b*
T0 (control)	68.38 ± 0.57 <sup>b</sup>	3.56 ± 0.13 <sup>a</sup>	25.82 ± 0.19 <sup>a</sup>
T1	74.49 ± 0.47 <sup>a</sup>	0.78 ± 0.04 <sup>b</sup>	20.46 ± 0.30 <sup>b</sup>
T2	74.17 ± 0.55 <sup>a</sup>	0.81 ± 0.11 <sup>b</sup>	20.56 ± 0.24 <sup>b</sup>
T3	74.22 ± 0.51 <sup>a</sup>	0.84 ± 0.08 <sup>b</sup>	20.41 ± 0.27 <sup>b</sup>
T4	73.96 ± 0.62 <sup>a</sup>	0.83 ± 0.05 <sup>b</sup>	20.79 ± 0.32 <sup>b</sup>
T5	73.71 ± 0.38 <sup>a</sup>	0.86 ± 0.09 <sup>b</sup>	20.75 ± 0.17 <sup>b</sup>
T6	74.04 ± 0.49 <sup>a</sup>	0.80 ± 0.07 <sup>b</sup>	20.47 ± 0.23 <sup>b</sup>
T7	73.85 ± 0.56 <sup>a</sup>	0.76 ± 0.13 <sup>b</sup>	20.59 ± 0.29 <sup>b</sup>
T8	73.96 ± 0.44 <sup>a</sup>	0.79 ± 0.05 <sup>b</sup>	20.30 ± 0.34 <sup>b</sup>

295 Values represent mean (n=3) ± SD. Different letters in each column represent significant difference at 5% level of  
 296 probability among samples.

### 298 3.5. Hardness of pasta

299 **Pasta's texture qualities are a crucial factor in determining whether or not customers will**  
 300 **ultimately accept the product**, and it is mainly considered as the most important qualitative aspect  
 301 of cooked pasta. **In terms of customers' perspectives**, high water absorption capacity, low cooking  
 302 loss and favorable texture (high hardness and low viscosity) can be considered as high cooking  
 303 quality (Luo, Wang et al. 2020, Zhang, Yu et al. 2023). The hardness of the **pastas were** examined  
 304 using a texture analyzer, and the results are shown in Figure 3. The control sample had the highest  
 305 hardness (6.16 N), and the hardness of low-protein and **cooked pastas** were significantly lower  
 306 than the control (p<0.05). Among the different low-protein pastas, the treatment containing 35%  
 307 PS+40% TS had the highest hardness. Overall, the hardness values of the **pastas** containing  
 308 starches were in the range of 4.43-5.20 N. The gluten present in wheat flour creates a strong  
 309 network in the **dough** and therefore the texture of the product containing gluten is desirable. So,  
 310 by removing gluten from the pasta formulation, the texture of the final product becomes more  
 311 fragile and its strength and hardness decrease (Yahyavi, Kamali Roustae et al. 2020). Hydrocolloids

312 are one of the most important additives used to improve the quality of gluten-free products, and  
 313 due to their gelling properties, they have a positive effect on the texture of gluten-free pasta and  
 314 improve the hardness of the texture (Chauhan, Saxena et al. 2017). In the present study, xanthan  
 315 gum was used to compensate for some of the textural changes due to the removal of semolina flour  
 316 and gluten. Jung and Yoon (Jung and Yoon 2017) also reported that the hardness of pastas  
 317 containing different levels of buckwheat, acorn and mung bean starches was lower than the control  
 318 pasta. Milde et al. (Milde, Chigal et al. 2020) also showed the improvement of the hardness and  
 319 strength of gluten-free **pastas** based on corn flour and cassava starch due to the addition of xanthan  
 320 gum. These researchers stated that the hydrophilic compounds of hydrocolloids react with proteins  
 321 through ionic charges and improve the structure of pasta. Yaseen and Shouk (Yaseen and Shouk  
 322 2011, Wang, Guo et al. 2022) found that incorporating high levels of corn starch into a low-protein  
 323 pasta formulation resulted in a brittle batter prone to breakage.

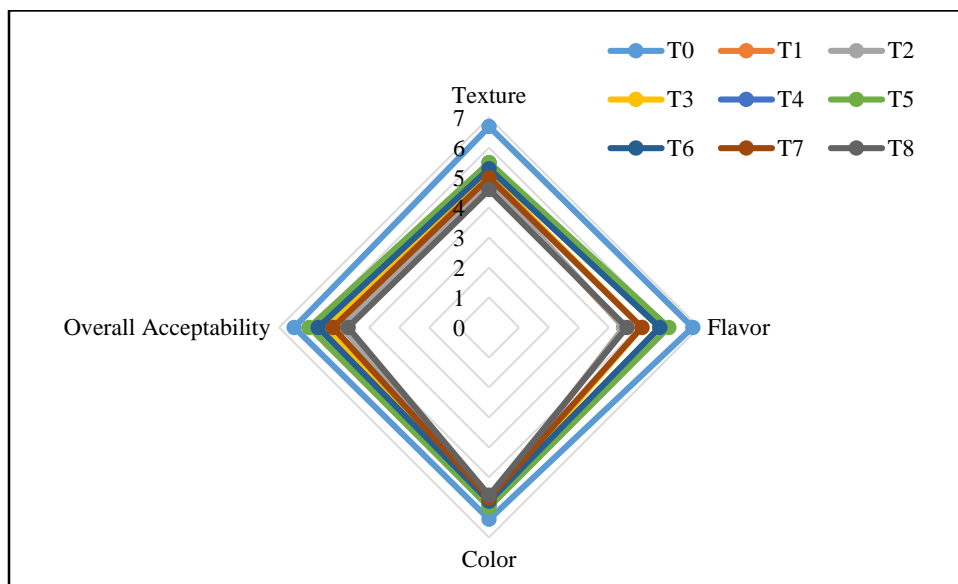


324  
 325 **Figure 3.** Hardness values (N) of **pastas**. Bars represent mean (n=3) ± SD. Different letters on the  
 326 bars indicate significant difference at 5% level of probability among samples.  
 327

### 328 3.6. Sensory evaluation of pastas

329 The sensory characteristics of **cooked pastas**, including texture, flavor, color and overall  
 330 acceptability, were evaluated using a 7-points Hedonic scales, and the results are shown in Figure  
 331 4. The control sample got the highest sensory scores, because this sample is the common and  
 332 commercial sample available in the market and its sensory characteristics are liked by consumers.  
 333 Substitution of semolina flour in the formulation of gluten-free pastas with the combination of PS  
 334 and TS caused a **decrease** in texture, flavor, color and overall acceptability scores of the produced  
 335 pasta compared to the control (p<0.05). In terms of texture, flavor and overall acceptability, except

336 for the treatments containing 75% PS, 75% TS and the combination of 65% PS+10% TS, which  
 337 received an average score, the other treatments had scored in the good to very good range. Despite  
 338 the decrease in the color score of the pastas compared to the control, **according** to the results, the  
 339 light color created in the pastas due to the replacement of wheat flour with the combination of PS  
 340 and TS was desirable and acceptable to consumers. Among the different pastas, the pasta  
 341 containing 35% PS+40% TS got the best overall acceptance score (6.00), and it was the best  
 342 treatment in terms of sensory. Yaseen and Shouk (Yaseen and Shouk 2011) observed that by  
 343 increasing the level of corn starch in the low protein pasta formulation, the sensory scores of the  
 344 samples **decreased**, however, the substitution of corn starch up to 60% was sensory acceptable.  
 345 Tao et al. (Tao, Wang et al. 2020) investigated the effect of different levels of PS on the sensory  
 346 properties of noodles and reported that noodles containing PS had high and favorable sensory  
 347 scores.



348  
 349 **Figure 4.** Sensory characteristics of **cooked** pasta.  
 350

#### 351 4. CONCLUSIONS

352 The results of this research showed that replacing semolina flour with the combination of PS and  
 353 TS significantly reduced the ash, protein and phenylalanine content of the pastas. The cooking  
 354 properties of pastas was also affected by the substitution of wheat flour with the combination of  
 355 starches, so that the cooking loss of low-protein pastas **were** higher than the control sample, but  
 356 they have less cooking time. Increasing the level of PS in the formulation also caused a further  
 357 decrease in cooking time. The color of low-protein and pastas was lighter than the control, but

358 their redness and yellowness was less. The replacement of wheat flour with the starches also made  
359 the texture of the pastas softer compared to control. The results of this study indicated that by using  
360 the combination of PS and TS instead of semolina flour, as well as xanthan gum and DKF, pasta  
361 with low phenylalanine can be produced for patients with PKU, and the produced pastas are also  
362 suitable and usable for celiac patients because they don't contain gluten. The best treatment in this  
363 research was the pasta containing 35% PS+40% TS, which had the best texture and the highest  
364 sensory scores.

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## تولید پاستای عملگرا با فنیل آلانین کم بر پایه نشاسته سیب زمینی و تاپیوکا

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علیرضا رضایی، مانیا صالحی فر، و وجیه فدایی نوغانی

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### چکیده

486

فنیل کتونوری (PKU) یک اختلال ژنتیکی است که نیاز به رژیم غذایی کم پروتئین و فنیل آلانین دارد. این مطالعه با هدف بررسی امکان سنجی تولید پاستای عملگرا کم پروتئین با استفاده از نشاسته سیب زمینی و تاپیوکا انجام شد. در فرمول پاستا، آرد سمولینا با مخلوطی از نشاسته سیب زمینی و تاپیوکا جایگزین شد. فیبر هسته خرما و صمغ زانتان به ترتیب به عنوان ترکیبات پری بیوتیک و تقویت کننده بافت ترکیب شدند. خواص فیزیکیوشیمیایی (رطوبت، چربی، خاکستر کل، پروتئین، فنیل آلانین، از دست دادن پخت، زمان پخت، شاخص های رنگ و سختی) و ویژگی های حسی (بافت، طعم، رنگ و مقبولیت کلی) با نمونه کنترل (بر اساس آرد سمولینا) مقایسه شدند. نتایج نشان داد که تغییر معنی داری در رطوبت و محتوای چربی پس از جایگزینی مشاهده نشد، اما کاهش معنی داری در محتوای خاکستر و پروتئین مشاهده شد ( $p < 0/05$ ). در نتیجه، سطح فنیل آلانین از 530.58 میلی گرم در 100 گرم در نمونه شاهد به 26.60-24.49 میلی گرم در 100 گرم در پاستا عملگرا کاهش یافت. جایگزینی آرد با نشاسته باعث افزایش تلفات پخت، کاهش زمان پخت و کاهش سختی پاستا نسبت به شاهد شد ( $p < 0/05$ ). پاستا حاصله  $L^*$  بالاتر و مقادیر  $a^*$  و  $b^*$  کمتری نسبت به شاهد نشان داد. ارزیابی حسی نشان داد که پاستا حاوی 35 درصد نشاسته سیب زمینی و 40 درصد نشاسته تاپیوکا بالاترین امتیاز را به دست آورد که نشان دهنده مقبولیت آن است. به طور کلی، این مطالعه نشان می دهد که ترکیب نشاسته سیب زمینی و تاپیوکا، همراه با فیبر هسته خرما و صمغ زانتان، تولید پاستا عملگرا و کم پروتئین مناسب برای بیماران PKU را امکان پذیر می کند.

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