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Chemical and Sensory Properties of New Gluten-free Food Products: Rice and Corn *Tarhana*

Erkan Yalçin[1], Süeda Çelik*, and Hamit Köksel

Department of Food Engineering, Hacettepe University, Beytepe, 06800 Ankara, Turkey

[1]Department of Food Engineering, Abant İzzet Baysal University, Gölköy, 14280 Bolu, Turkey

Abstract New gluten-free food product (*tarhana*) was produced using rice and corn flours. Chemical and sensory properties of the *tarhana* samples were investigated and compared with those of traditional wheat *tarhana*. Generally, sensory analysis results indicated that utilization of corn and rice flours in *tarhana* resulted in acceptable soup properties in terms of some of the sensory properties. The changes in electrophoretic properties of the proteins of the *tarhana* samples were also studied during the *tarhana* production. Sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE) results showed that relative intensities of some of the protein bands in *tarhana* samples generally decreased during fermentation. The decrease was more obvious at the larger molecular weight region. Corn and rice *tarhana* seem to be promising food products for the celiac patients who have limited choice of cereal based foods.

Key words: celiac disease, *tarhana*, rice, corn, sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE)

Introduction

Celiac disease, also known as gluten-sensitive enteropathy, is an autoimmune disease that affects the small intestine. People with celiac disease cannot tolerate gluten, a protein that is found in grains and contains a specific amino acid sequence. It is generally accepted that the cereals: wheat, rye, barley, and possibly oats or their prolamines, gliadin, hordein, secalin, and avenin respectively, are the major triggering factors in celiac disease (1,2). Rice and corn do not initiate celiac disease. Exposure to gluten initiates an immune response in the intestine. The villi become flattened and inflamed causing malabsorption (3). Once gluten is removed from the diet, the villi return to the normal state. However, any time that gluten is consumed, the harmful immunologic responses reoccur. Treatment for celiac disease requires lifetime strict adherence to a gluten-free diet. Foods containing these proteins are very common. Adhering to a gluten-free diet is extremely difficult and affects the lifestyles of individuals with celiac disease to a large extent (2,4). Hence a variety of foods should be developed to meet the rising demand for gluten-free products.

Fermented cereal-yoghurt mixtures play an important role in the diets of many people in the Middle East, Asia, Africa, and some parts of Europe (5). *Tarhana*, a popular traditional fermented food product in Turkey, is prepared by mixing yoghurt, wheat flour, yeast, and a variety of vegetables and spices followed by fermentation for 1-7 days. Lactic acid bacteria and yeast are responsible for the acid formation during fermentation. After fermentation the mixture is dried and ground. *Tarhana* has an acidic and sour taste with a yeasty flavor and is used for soup preparation (6). Because of the low moisture content (about 10%) and low pH, it can be stored for long periods of time (7). There are some other products similar to *tarhana* such as *kishk* in Egypt, *kushuk* in Iraq, and *tahonya/talkuna* in Hungary and Finland (8,9). Methods for preparation for such mixtures may vary, but cereals and yoghurt are always the two major components. The amount and type of ingredients used in *tarhana* production may affect its nutritional content and sensory attributes (10). In general, *tarhana* is produced with white wheat flour. Whole meal flour or semolina can also be used (11). However, gluten in these sources makes *tarhana* unsuitable for celiac patients.

The objectives of this study were to produce new gluten-free food products (*tarhana*) using rice and corn flour and investigate their chemical and sensory properties. Effect of *tarhana* production on the electrophoretic properties of proteins was also evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and Methods

Materials Wheat, corn, and rice flours, yoghurt, fresh baker's yeast, tomato paste, green and red peppers, onion, salt, and paprika were purchased from local markets in Ankara, Turkey.

Preparation of tarhana *Tarhana* samples were prepared according to the method of Ibanoglu *et al.* (10) with some modifications. The ingredients used in *tarhana* preparation are given in Table 1. To prepare *tarhana* samples, onions, green and red peppers were chopped in a food processor (MR 1001; Raks, Ankara, Turkey). Tomato paste, red pepper, and salt were added and the mixture was blended. Flour, yoghurt, and yeast were added to the mixture and blended until homogenization. The resulting mixture was taken into covered containers and fermented at 30°C for 5 days. Samples were taken initially, during the course of the fermentation and at the end of the fermentation for the electrophoretic analysis. After fermentation, the *tarhana*

*Corresponding author: Tel: +90-312-297-7109; Fax: +90-312-299-2123
E-mail: sueda@hacettepe.edu.tr
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Table 1. Recipe used for preparation of *tarhana* samples

Ingredient	Amount[1)] (g)
Flour[2)]	500
Yoghurt (from cow's milk)	400
Tomato paste	75
Green pepper	50
Red pepper	50
Onion	120
Yeast	10
Paprika	10
Salt	40
Total	**1,255**

[1)]As is basis.
[2)]Wheat, corn, or rice flour.

was dried at room temperature and then ground and sieved to pass a 1-mm screen to obtain soup base for preparing *tarhana* soup.

Analytical methods Moisture, ash, and crude fat contents of flour and *tarhana* samples were determined according to American Association of Cereal Chemists (AACC) methods (12). Nitrogen content of the samples were determined by Kjeldahl method (12) and converted to protein content by a factor of 5.7 for wheat, 6.25 for corn, and 5.95 for rice samples. pH was determined according to the method of Ibanoglu *et al.* (10). Acid formation in *tarhana* samples was determined according to Turkish *Tarhana* Standard (11) and expressed as a percent of total lactic acid.

SDS-PAGE analysis Protein extraction and SDS-PAGE was performed according to the method of Ng and Bushuk (13). During the *tarhana* fermentation (1-5 days), a small amount of sample was removed each day and then lyophilized in order to investigate the effects of fermentation on proteins by using SDS-PAGE. The flours, lyophilized samples, and *tarhana* samples (80 mg) were dissolved in 1.0 mL of buffer solution (pH 6.8) containing 0.063 mol/L Tris-HCl, 2%(w/v) SDS, 7%(v/v) 2-mercaptoethanol, 20%(w/v) glycerol, and 0.01%(w/v) Pyronin Y. The *tarhana* samples (10 μL), yoghurt, and skim milk (3 μL) were applied to the SDS-PAGE which was carried out in a cooled slab gel unit (Hoefer Scientific Instruments, San Fernando, CA, USA). The gels were stained overnight with Coomassie Brillant Blue G-250 according to Ng and Bushuk (13). Apparent molecular weights were estimated using the wide range molecular weight markers (Sigma-Aldrich, St. Louis, MO, USA).

Sensory analysis Soups made from the wheat, corn, and rice *tarhana* samples were subjected to sensory evaluation (14). Seven people who were familiar with *tarhana* were asked to score the *tarhana* soups in terms of color, taste, odor, mouthfeel, and consistency using a 5-point scale, with 1 being 'dislike extremely' and 5 being 'like extremely'. *Tarhana* soups were prepared by mixing 40 g soup base sample with 500 mL water and simmering for 10 min with constant stirring. The cooked samples were served to the panelists at 80°C in random order.

Pasting properties Starch pasting properties of flour and *tarhana* samples were measured using a rapid visco-analyzer (RVA-4; Newport Scientific, Warriewood, NSW, Australia) with data analysis software (Thermocline). Distilled water (25 g) was added to a 4.0 g ground sample weighed on a 14% moisture basis. The RVA pasting curve was obtained by using the following test profile; initial equilibrium at 50°C for 1 min 20 sec, heating to 95°C over 3 min 25 sec, holding at 95°C for 2.5 min, cooling to 50°C over 3 min 45 sec, and holding at 50°C for 2 min.

RVA™ soup method A rapid visco-analyzer (RVA-4; Newport Scientific,) with data analysis software (Thermocline) was used to analyze soup samples. The last viscosity value of *tarhana* soups prepared in this study has been determined by RVA soup method, cooled sample profile (15). Details of cooled sample profile are as follows: initial equilibrium at 80°C, holding at 80°C for 5 min.

Color measurement The color of *tarhana* samples was measured using the L*a*b* color space (CIELAB space) with a spectrophotometer (CM-3600d; Minolta, Osaka, Japan). The L* value indicates lightness, the a* and b* values are the chromaticity coordinates (a* from green to red; b* from blue to yellow).

Statistical analysis The data were statistically evaluated by the 1-way analysis of variance (ANOVA) procedure using the SPSS statistical programme. When significant differences were found, the least significant difference (LSD) test was used to determine the differences among means.

Results and Discussion

Chemical characteristics of flour and *tarhana* samples Chemical properties of wheat, corn, and rice flour samples are presented in Table 2. The ash content of corn flour was lower than the other samples used (0.38%), while wheat flour had the highest ash value (0.52%). The wheat flour had the highest protein content of 13.0% and corn flour had the lowest protein content of 5.9%. The fat content of the samples ranged from 0.92 to 1.27%.

Chemical characteristics of *tarhana* samples are also presented in Table 2. The moisture content of *tarhana* samples varied between 9.7 and 10.4%. It was previously reported that the variation in moisture content of *tarhana* samples was due to the properties of ingredients used in the formulation and the drying method (16). The ash contents of *tarhana* samples were between 0.87 and 1.51%. Corn *tarhana* sample had the lowest ash content while the wheat *tarhana* had the highest value as expected from the ash contents of respective flour samples. The composition of *tarhana* sample was affected by ingredients, especially flour.

Corn *tarhana* had the lowest protein content (9.5%) while the wheat *tarhana* had the highest protein content (17.3%). It has been reported in some studies that the main reason for variation in protein content of *tarhana* may be the type and amount of yoghurt used in *tarhana* preparation (16,17). Besides these, the properties of different cereal and legume flour samples could also affect the protein content

Table 2. The chemical composition of flour and *tarhana* samples[1)]

Sample	Moisture[2)] (%)	Ash[3)] (%)	Protein[3)] (%)	Fat[3)] (%)	Acidity (%)	pH[2)]
Wheat flour	11.9±0.07	0.52a	13.0a	1.23a	-	-
Corn flour	12.6±0.09	0.38c	5.9c	1.27a	-	-
Rice flour	10.6±0.02	0.49b	6.9b	0.92b	-	-
Wheat *tarhana*	9.7±0.10	1.51a	17.3a	4.53a	1.4	4.51±0.057
Corn *tarhana*	10.4±0.01	0.87c	9.5c	4.57a	1.4	4.48±0.099
Rice *tarhana*	10.1±0.01	1.35b	11.9b	4.33b	1.5	4.48±0.255

1)Means with the same letter within a column are not significantly different by least significant difference (LSD) analysis ($p<0.05$).
2)Mean±SD.
3)Dry basis.

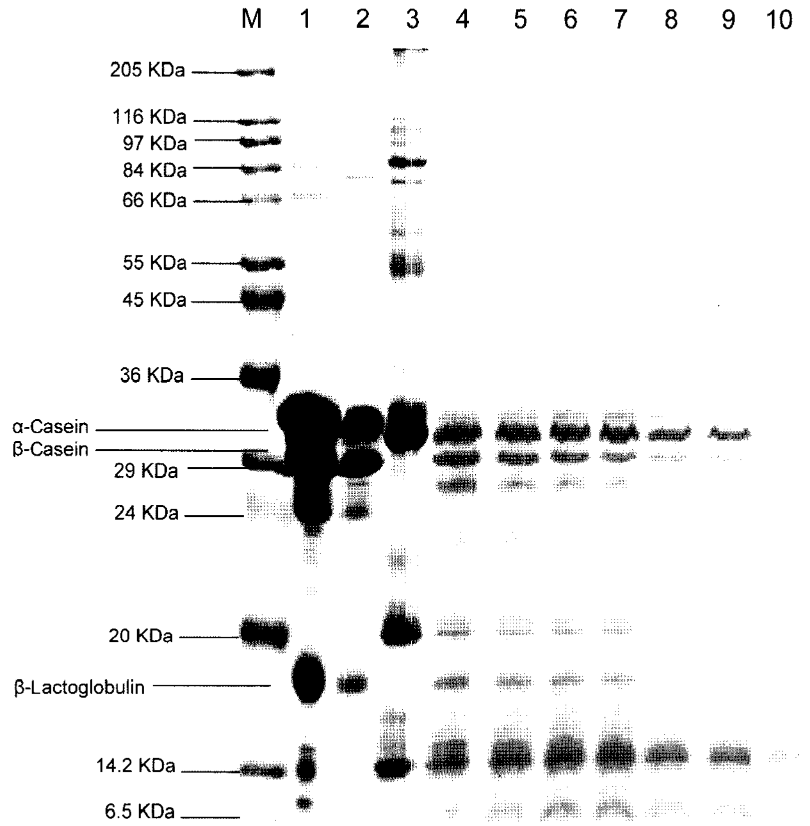

Fig. 1. SDS-PAGE patterns of rice *tarhana* samples. M, Wide-range protein markers; 1, skim milk powder; 2, yoghurt; 3, rice flour; 4-9, *tarhana* samples taken at different fermentation periods; 0th, 1st, 2nd, 3rd, 4th, and 5th day, respectively; 10, rice *tarhana* (end product).

(14,18,19). Since the type and amount of yoghurt samples used in this research were the same for all *tarhana* samples, it can be concluded that the reason for the variation of protein contents is the type of flour samples used in *tarhana* preparation. Both wheat and corn *tarhana* had higher fat content of around 4.5% and rice *tarhana* had lower fat content of 4.33%. The slight differences in fat contents of *tarhana* samples are probably due to the different fat contents of the flour samples used in the formula. All *tarhana* samples had comparable acidity values between 1.4 and 1.5% and pH values between 4.48 and 4.51.

SDS-PAGE patterns of flour and *tarhana* samples SDS-PAGE patterns of rice flour, skim milk powder, yoghurt, and *tarhana* samples as well as the specimens taken during the course of the fermentation (0-5 days) are presented in Fig. 1. Effect of *tarhana* production on the electrophoretic properties of proteins during the course of the fermentation was first time evaluated in this study by using SDS-PAGE. Skim milk powder and yoghurt was used to compare their proteins (α- and β-caseins, β-lactoglobulin) with the ones of respective *tarhana* samples in electrophoregrams. In this electrophoregram, milk proteins were termed as described in Havea (20). Relative band intensities of the protein bands of *tarhana* mixture at the beginning of fermentation (0th day) were generally less intense than those of the rice flour sample. The reduction in relative band intensities might be due to relative dilution of flour proteins by addition of other *tarhana* ingredients. Relative band intensities of certain protein bands decreased during the fermentation (Fig. 1, lane 3-8). The reduction in relative band intensities might be due to the hydrolysis of proteins during fermentation (0-5 days). The decrease was more obvious at

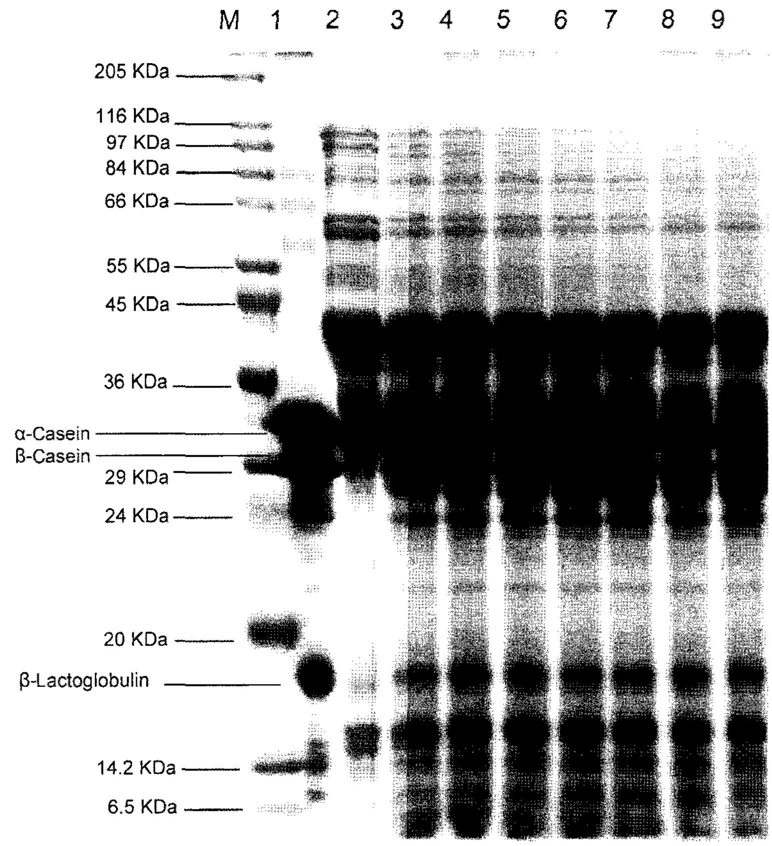

Fig. 2. SDS-PAGE patterns of wheat *tarhana* samples. M, Wide-range protein markers; 1, skim milk powder; 2, wheat flour; 3-8, *tarhana* samples taken at different fermentation periods; 0^{th}, 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} day, respectively; 9, wheat *tarhana* (end product).

the larger molecular weight region (45-116 kDa) and also in the range of 18-36 kDa Mw. It is suggested in previous studies that lactic acid bacteria can hydrolyze the proteins in the fermentation medium by their proteolytic enzymes (16,21,22). Furthermore, some protein bands were observed in the electrophoregrams of *tarhana* samples (Fig. 1, lane 4-10) around 29, 24, and 18 kDa which did not exist in the flour sample. These protein bands are thought to be the α- and β-caseins and β-lactoglobulin proteins of the yoghurt sample since they had same relative mobilities with the proteins in skim milk and yoghurt used for comparison.

SDS-PAGE patterns of wheat flour, skim milk powder, and *tarhana* samples are presented in Fig. 2. The *tarhana* samples included the dried final product as well as the specimens taken during the course of the fermentation (0-5 days). Similar to the rice *tarhana* electrophoresis results, relative band intensities of corn *tarhana* (electrophoregram not presented) and wheat *tarhana* samples (Fig. 2, lane 3-9) also generally decreased during fermentation. The decrease was more obvious especially at the larger molecular weight region (45-116 kDa). The overall electrophoresis results indicated that molecular weights of proteins decreased during fermentation, regardless of cereal source.

Sensory analysis Sensory analysis results of the soups made from the *tarhana* samples are presented in Table 3. The effect of different cereal flours on the color, taste, and odor of *tarhana* soups was statistically significant ($p<0.05$). Color values obtained by sensory analysis of the *tarhana* soups varied between 3.29 and 4.14. The color values of rice *tarhana* soups were not significantly different from that of wheat *tarhana* soup. Taste values of *tarhana* soups were between 2.86 and 4.00. The sensory properties of corn and rice *tarhana* soups were generally comparable. The effects of different flours on mouthfeel and consistency values of *tarhana* soups were not statistically significant. The results of the sensory analysis showed that utilization of rice and corn flours in *tarhana* preparation resulted in acceptable soup properties in terms of some of the sensory properties. It was reported that sensory evaluation of *tarhana* samples produced using other cereals gave relatively lower scores in comparison with the traditional

Table 3. Sensory analysis results of *tarhana* soups[1)]

Tarhana sample	Color	Taste	Odor	Mouthfeel	Consistency
Wheat *tarhana*	4.14a	4.00a	4.29a	3.86	4.14
Corn *tarhana*	3.29b	2.86b	3.29b	3.14	3.57
Rice *tarhana*	3.86ab	3.14b	3.29b	3.00	3.29

[1)]Means with the same letter within a column are not significantly different by least significant difference (LSD) analysis ($p<0.05$).

Table 4. RVA pasting properties of flour and *tarhana* samples[1)]

Sample	Peak 1 (cp)	Trough1 (cp)	Breakdown (cp)	Final viscosity (cp)	Setback (cp)
Wheat flour	3625c	2308b	1317c	3838c	1530c
Corn flour	5464b	3298a	2166b	7191a	3893a
Rice flour	8016a	3305a	4711a	5745b	2440b
Wheat *tarhana*	2520c	1388b	1131	2414c	1026b
Corn *tarhana*	3402a	2313a	1089	3978a	1666a
Rice *tarhana*	3000b	2236a	764	3232b	995b

[1)]Means with the same letter within a column are not significantly different by least significant difference (LSD) analysis (p<0.05).

wheat *tarhana* (14,18,19). However, sensory quality of soups prepared from rice and corn flours can be improved by further studies. Different *tarhana* recipes should be tried to improve the sensory properties of rice and corn *tarhana* in order to reach the traditional sensory properties of *tarhana* prepared from wheat flour.

RVA pasting properties of flour and *tarhana* samples RVA pasting properties of wheat, corn, and rice flours and their respective *tarhana* samples are presented in Table 4. There were significant differences in the RVA viscosity values of wheat, corn, and rice flours samples. The highest peak, trough, and breakdown values were obtained for the rice flour sample and the lowest peak, trough, and breakdown viscosity values were obtained for the wheat flour.

There were significant differences in the RVA viscosity values of wheat, corn, and rice *tarhana* samples. The highest and lowest peak, trough, and final viscosity values were obtained for the corn and wheat *tarhana* samples, respectively. Corn and rice *tarhana* samples had favorable hot paste stability (low RVA breakdown values) relative to the wheat *tarhana* sample, which might be desirable in soup products. The viscosities of these samples are not expected to decrease drastically during the prolonged cooking process since they are affected from sheer thinning to a lower level.

RVA soup method The last viscosity values (RVA soup index) of *tarhana* soups are presented in Table 5. Viscosity is an important quality criterion in soups. The viscosity values of *tarhana* soups showed variation between 400 and 834 cp. RVA soup index values of corn and rice *tarhana* samples were comparable and significantly different from that of wheat *tarhana*. Since different cereal flours used in *tarhana* preparation vary in their water absorption/pasting properties (Table 4), last viscosity values of the soups can be different from each other. This might be due to their differences in starch characteristics. Although there were some statistically significant differences in the RVA soup index values of different *tarhana* samples, the higher RVA soup index values of corn and rice *tarhana* are not expected to cause substantial problems in the related sensory properties (mouthfeel and consistency). The soup samples were cooked with the same amount of *tarhana* powder and water level to aid comparison and these soups could be diluted during domestic soup preparation to obtain a thinner soup and improve sensory properties in terms of mouthfeel and consistency.

Table 5. RVA soup index values of *tarhana* samples[1)]

Sample	RVA soup index (cp)
Wheat *tarhana*	400b
Corn *tarhana*	834a
Rice *tarhana*	775a

[1)]Means with the same letter are not significantly different by least significant difference (LSD) analysis (p<0.05).

Table 6. Color analysis of flour and *tarhana* samples[1)]

Sample	L*	a*	b*
Wheat flour	93.55a	0.44b	9.14b
Corn flour	91.65b	3.12a	22.98a
Rice flour	93.43a	-0.36c	7.02c
Wheat *tarhana*	79.98b	13.75a	27.85b
Corn *tarhana*	82.22a	12.41b	29.69a
Rice *tarhana*	82.15a	13.19ab	26.26b

[1)]Means with the same letter within a column are not significantly different by least significant difference (LSD) analysis (p<0.05); L, whiteness; a*, redness and greenness; b*, yellowness and blueness.

Color measurements The color of *tarhana* samples measured using the L*a*b* color space (CIELAB space) are presented in Table 6. The differences between color values of *tarhana* samples were significant. The L* values of corn *tarhana* and rice *tarhana* samples were comparable and higher than that of wheat *tarhana*. The higher L* values of corn and rice *tarhana* samples indicate that they have brighter color than that of wheat *tarhana*. Redness, a*, was recorded between 13.75 and 12.41 and yellowness, b*, was found to be between 29.69 and 26.26. The b* value of corn *tarhana* was the highest as compared to the other samples probably due to more evident yellow color of the corn flour.

Corn and rice are suitable for a wide range of food applications and they can be processed into a number of pleasant, nutritious food products. They are also safe to use in gluten-free products. However, to the best of our knowledge there are no studies on the *tarhana* production for celiac patients. Hence, in this study corn and rice flours were utilized in *tarhana* formulation in order to produce new gluten-free food products. The results of the sensory analysis showed that utilization of corn and rice flours in *tarhana* preparation resulted in acceptable soup properties in terms of most of the sensory properties. Slightly lower values in some of the sensory properties (color and taste) of the corn and rice *tarhanas* might decrease the acceptability of these *tarhana* soups as compared to traditional wheat based *tarhana*. However, the slightly lower acceptability can be compensated by the health benefits to celiac patients and sensory quality of soups prepared from corn and rice flours can be improved by further studies.

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