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Effects of Fermentation Temperature and Time on the Physicochemical and Sensory Characteristics of Douchi

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Summary

In this paper studies were made to generate data on the physicochemical and sensory characteristics of douchi prepared by fermentation at different temperatures for variable time prior to mechanical drying. Fermentation was carried out at 20, 30 and 40 °C for 12, 24, 48 and 72 h prior to drying at 50 °C. Physiochemical and sensory characteristics were monitored. The results show that fermentation conditions of 30 °C for 24 h and 30 °C for 48 h bring about more acidic douchi with superior sensory characteristics.

Key words: fermentation of douchi, sensory characteristics

Introduction

Douchi is important legume based traditional fermented food in China. It has been used as a savoury by most of the vegetarians as a substitute for meat (*1*). Douchi is a product similar to Indian wari and is in the form of brittle and spongy textured dried balls ranging from 2–5 cm in diameter. It is usually prepared in cottage or home scale level and used as an additive in curry after deep fat frying or boiling in curry (*1,2*).

Douchi is commonly prepared from split blackgram (*Phaseolus mungo*) or greengram (*Phaseolus aureus*) and colocasia (seppan kizhangu) tuber, ashgourd (*Benincasa hispida*) or radish (*Raphanus sativus*) depending on the availability (*3*).

Douchi is a rich source of protein (18–20 %), carbohydrates (67–70 %) and minerals. The protein quality of douchi is comparable to animal proteins (*2*) such as lean meat, which contains 11–20 % protein (*4*).

The preparation of douchi involves sun drying of initial dough for 3 to 5 days. The quality of douchi depends on the effective sun drying; rainy seasons often result in spoilage. Controlled fermentation and mechanical drying seem to be a potential way to commercialize the douchi product. In this study an attempt has been made to generate data on the physicochemical and sensory characteristics of douchi prepared by fermentation at different temperatures for variable time prior to the mechanical drying since such information is not sufficient in spite of its popularity on the industrial scale.

Materials and Methods

Blackgram (*Phaseolus mungo*) was collected from the local market of Nanning, China. Colocasia (seppan kizhangu) tuber was purchased from the local market of Wuxi, China.

Preparation of douchi

Dough

Cleaned blackgram was soaked in water (mass ratio=1:4) for 16 h at ambient conditions. The soaked blackgram was hand washed in water (mass ratio=1:2) 2–3 times. The dehusked blackgram was drained for 10 min and then ground into a thick paste (~70 % mois-

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ture). On the other hand, colocasia tuber was washed, peeled, sliced and subjected to wet grinding using water (mass ratio=5:2) to get a thick paste (~85 % moisture). The moisture of the colocasia paste was maintained at about 70 % using colocasia powder (obtained after peeling the colocasia tuber, slicing, blanching at 70 °C for 5 min, drying at 55 °C for 16 h, grinding and sieving at 80 meshes). The blackgram paste and colocasia paste were then mixed homogeneously at the mass ratio of 1:1 for 5 min.

Controlled fermented douchi

The dough is then made into small lumps weighing 20–30 g each and placed on trays 1–2 inches apart. Trays were then subjected to fermentation in the incubator at three different temperatures at 20, 30 and 40 °C, respectively, and under constant humidity of 80–90 %. Initial dough was also subjected to analysis within 48 h of storage in the freezer.

Traditional douchi

Another set of trays was also subjected to sun drying for 5 days (at 20 °C, and humidity 70 %) for the preparation of traditional douchi. The trays from the incubator were taken out after certain period of fermentation time, *i.e.* 12, 24, 48 and 72 h and then subjected to drying at 50 °C for 12–16 h. The dried product was grinded and sieved at 60 meshes and used for the analysis.

Analytical methods

Approximate composition

Moisture, ash, fat and fiber were estimated by standard AOAC (*5*) method. Total protein was estimated directly in the digested extract of sulphuric acid (15 mL of sulphuric acid, 5 g of digestion mixture and 1 g of sample powder digested at 420 °C for 70 min and cooled to the room temperature) in the automatic distillation and titration instrument (Kjeltec 2300 analyzer unit, Foss Tecator AB, Sweden) on the basis of micro-Kjeldahl principle.

Acidity and pH

Total acidity was calculated in terms of lactic acid by titrating against 0.1 M sodium hydroxide according to AOAC (*5*) method, and pH was measured directly using a pH meter (Leici Instrument Company, Shanghai, P.R. China).

Soluble protein and amino nitrogen

Soluble protein was determined according to the method of Mattil (*6*). One gram of powder (or 5 g in case of dough) was mixed with 15 mL of water and pH was adjusted to pH=7.0 by the addition of alkali or acid. The volume was then adjusted to 20 mL with water. It was shaken for 1 h at room temperature and centrifuged at 5000 rpm for 20 min. The supernatant was used for protein estimation. Amino nitrogen was determined by the modified Sorensen's method as described in AACC (*7*). The results were expressed as mg of amino nitrogen per 100 g of sample.

Total free sugar and reducing sugar

Free sugar in the sample was extracted by 70 % ethanol. Total carbohydrate and reducing sugar in the extract were estimated by the phenol-sulphuric acid method of Dubois *et al*. (*8*) and DNSA method, respectively (*9*).

Organoleptic evaluation

Organoleptic evaluation of douchi samples was done using the 5-point hedonic scale. Douchi samples were boiled in water (mass ratio=1:5) with 0.5 % NaCl for 10 min and the douchi curry was evaluated according to the criteria given by a panel composed of 10 trained judges as follows:

- excellent (5): pleasant smell, pleasant taste with good flavour, no bitterness
- very good (4): sour but pleasant taste with good flavour, a little bitter
- good (3): moderate flavour, moderate taste and moderately sour
- average (2): less flavour, less taste and less sour
- poor (1): putred flavour, off smell and bitter taste.

Statistical analysis

All determinations were carried out at least twice and the mean and standard deviation for each of the determinations were calculated and reported. For the sensory scores, two-factor (time and temperature) analysis was carried out after two-way ANOVA for the factorial design followed by the mean separation technique within each factor (*10*).

Results and Discussion

Chemical composition of the blackgram and colocasia tuber, used in the preparation of douchi, was analyzed and given in Table 1. Blackgram was found to be a good source of proteins (27.3 %) and minerals (3.9 %), whereas colocasia was the major source of carbohydrates (78.6 %) and minerals (4.4 %).

Table 1. Chemical composition of the raw materials used in douchi preparation

Parameters	Blackgram	Colocasia tuber
w(moisture)/%	11.60±0.04	76.50±0.30
Acidity as lactic acid/%	1.08±0.13	0.87±0.09
pH	6.25±0.03	6.45±0.03
w(total sugar as glucose)/%	5.00±0.33	8.50±0.53
w(total reducing sugar as glucose)/%	1.10±0.16	2.90±0.07
w(total protein N_2×6.25)/%	27.30±0.17	13.14±0.23
w(soluble protein)/%	60.80±0.04	47.60±1.37
w(amino nitrogen)/(mg/100 g)	3.60±0.35	5.10±0.29
w(total ash)/%	3.90±0.06	4.40±0.15
w(total fat)/%	1.71±0.06	1.10±0.06
w(total fiber)/%	2.50±0.30	2.80±0.40
w(total carbohydrate by diff.)/%	64.50	78.60

Results are the means of the three determinations±S.D.
Values except moisture are given on dry basis

Acidity, pH, free sugar, reducing sugar, soluble protein and amino nitrogen content of the two raw materials were also analyzed and the results are given in Table 1. The values are comparable to the previously reported values (*11*).

Biochemical changes during the preparation of douchi by both controlled fermentation and by traditional method were assessed in relation to its raw materials. The biochemical changes that occur during the preparation of traditional douchi are given in Table 2.

Acidity as lactic acid/%, soluble protein as *w*(total protein)/%, *w*(amino nitrogen)/(mg/100 g) and *w*(total ash)/% values were found to increase significantly (p=0.05) from 0.87 to 1.82; from 53.9 to 69.4; from 4.92 to 9.46 and from 3.61 to 4.89, respectively, during sun drying of the traditional douchi for 5 days. Simultaneously, free sugar as *w*(glucose)/% and reducing sugar as *w*(glucose)/% values were found to decrease from 6.77 to 5.31 and 2.59 to 0.92, respectively. Total protein content did not show any significant changes during sun drying and it ranged from 18.85 to 19.62 % on dry basis.

Biochemical changes caused during the preparation of douchi by controlled fermentations at 20, 30 and 40 °C and mechanical drying are given in Tables 3, 4 and 5, respectively. Fractions of soluble protein, amino nitrogen and total ash values also increased constantly from 48.6 to 59.9 %, from 5.68 to 8.77 mg/100 g and from 4.33 to 5.92 %, respectively, during 72 h of fermentation. Fraction of total protein slightly increased from 19.41 to 22.1 %. The value of pH constantly decreased from 6.38 to 5.02 during 72 h of fermentation. Fractions of free sugar and reducing sugar also decreased from 7.63 to 2.15 % and from 2.71 to 0.86 %, respectively, during 48 h of fermentation, while they slightly increased to 6.52 % for free sugar and 1.26 % for reducing sugar after 72 h of fermentation (Table 3).

Table 2. Biochemical changes during sun drying of traditional douchi

Parameters	Initial dough*	TM**
Acidity as lactic acid/%	$0.87^{a}\pm0.04$	$1.82^{b}\pm0.05$
w(total free sugar as glucose)/%	$6.77^{a}\pm0.46$	$5.31^{b}\pm0.37$
w(total reducing sugar as glucose)/%	$2.59^{a}\pm0.54$	$0.92^{b}\pm0.15$
w(total protein $N_2\times6.25$)/%	$18.85^{a}\pm0.11$	$19.62^{a}\pm0.72$
w(soluble protein)/%	$53.90^{a}\pm1.10$	$69.40^{b}\pm4.90$
w(amino nitrogen)/(mg/100 g)	$4.92^{a}\pm0.02$	$9.46^{b}\pm0.27$
w(total ash)/%	$3.61^{a}\pm0.21$	$4.89^{b}\pm0.03$

*initial dough prepared by mixing the blackgram paste with the colocasia paste
**TM indicates the traditional douchi prepared by sun drying
Results are the mean of the triplicates±S.D. and on dry basis.
Means marked with different letters (a, b) in the same row indicate the significant (p<0.05) difference

At 30 °C, acidity values were also increasing constantly from 0.87 % to the max. of 3.25 % after 48 h of fermentation time but the acidity was reduced to 0.22 % after 72 h of fermentation. Similarly, pH value decreased from 6.31 to 4.74 after 48 h of fermentation time and increased to pH=6.85 after 72 h of fermentation time. Fractions of soluble protein and amino nitrogen values increased constantly from 54.01 to 65.6 % and from 6.94 to 8.50 mg/100 g after 48 h of fermentation but slightly decreased afterwards to 59.17 % and 6.11 mg/100 g, respectively. Fractions of total free sugar and reducing sugar were found to decrease constantly from 9.63 to 4.87 % and from 3.49 to 0.21 % after 72 h of fermentation. Fractions of total protein slightly increased from 18.46 to 23.29 % on dry basis (Table 4). Fractions of total ash increased from 4.44 to 5.51 % after 72 h of fermentation at 30 °C.

At 40 °C similar trend was observed in the changes of the biochemical parameters. Acidity values ranged from 0.83 to 2.47 % after 24 h of fermentation and the values again decreased to 1.49 % after 48 h and to 0.29 % after 72 h. Similarly, pH value decreased from 6.23 to 4.93 after 24 h and then increased to 6.27 after 72 h. Fractions of soluble protein slightly increased from 49.01 to 55.36 % after 72 h. Amino nitrogen values constantly increased from 6.33 to 8.76 mg/100 g after 24 h and then were slightly reduced to 7.14 mg/100 g. Fractions of free sugar and reducing sugar values constantly decreased from 9.19 to 5.04 % and from 4.96 to 0.26 % after 72 h, respectively. Fractions of total ash increased from 4.52 to 5.78 after 72 h fermentation at 40 °C.

Among these 3 fermentation temperatures, the highest acidity values of douchi samples of about 3.25 % after 48 h of fermentation and simultaneously the lowest

Table 3. Biochemical changes during the fermentation of douchi at 20 °C

Parameters	Dough	0 h	12 h	24 h	48 h	72 h*
Acidity/%	$0.82^{a}\pm0.02$	$1.05^{b}\pm0.02$	$1.17^{c}\pm0.05$	$1.40^{d}\pm0.12$	$1.41^{d}\pm0.09$	$1.96^{e}\pm0.14$
pH	$6.38^{a}\pm0.02$	$6.01^{b}\pm0.03$	$5.85^{c}\pm0.03$	$5.68^{d}\pm0.01$	$5.60^{e}\pm0.03$	$5.02^{f}\pm0.02$
w(free sugar as glucose)/%	$7.63^{a}\pm0.21$	$6.96^{a}\pm1.21$	$7.44^{a}\pm0.41$	$5.05^{b}\pm0.65$	$2.15^{c}\pm0.60$	$6.52^{d}\pm0.43$
w(reducing sugar as glucose)/%	$2.71^{a}\pm0.21$	$2.38^{b}\pm0.13$	$2.07^{c}\pm0.36$	$1.48^{d}\pm0.09$	$0.86^{e}\pm0.21$	$1.26^{f}\pm0.05$
w(total protein $N_2\times6.25$)/%	$19.41^{a}\pm0.27$	$20.38^{a}\pm0.02$	$20.37^{a}\pm0.11$	$20.69^{a}\pm0.16$	$21.96^{b}\pm0.03$	$22.10^{b}\pm0.87$
w(soluble protein)/%	$48.60^{a}\pm2.40$	$53.20^{b}\pm0.54$	$57.10^{c}\pm0.05$	$58.90^{c}\pm1.98$	$59.10^{c}\pm0.04$	$59.90^{c}\pm1.00$
w(amino nitrogen)/(mg/100 g)	$5.68^{a}\pm0.88$	$7.26^{b}\pm0.54$	$6.17^{b}\pm1.13$	$7.29^{c}\pm0.01$	$6.95^{c}\pm0.47$	$8.77^{d}\pm0.01$
w(total ash)/%	$4.33^{a}\pm0.05$	$4.82^{b}\pm0.15$	$4.86^{b}\pm 0.12$	$5.15^{b}\pm0.20$	$5.53^{b}\pm0.35$	$5.92^{b}\pm0.17$

Results are the mean of triplicates±S.D. and on dry basis
*indicates the appearance of surface mould on the product
Means marked with different letters (a, b, c, d, e, f) in the same row indicate the significant (p<0.05) difference

Table 4. Biochemical changes during the fermentation of douchi at 30 °C

Parameters	Dough	0 h	12 h	24 h	48 h	72 h*
Acidity/%	$0.87^{a}\pm0.04$	$1.08^{b}\pm0.07$	$1.53^{c}\pm0.04$	$2.39^{d}\pm0.12$	$3.25^{e}\pm0.12$	$0.22^{f}\pm0.05$
pH	$6.31^{a}\pm0.01$	$6.04^{b}\pm0.04$	$5.56^{c}\pm0.04$	$4.83^{d}\pm0.01$	$4.74^{d}\pm0.06$	$6.85^{e}\pm0.11$
w(free sugar as glucose)/%	$9.63^{a}\pm0.44$	$8.57^{b}\pm1.05$	$8.25^{a}\pm0.32$	$6.81^{b}\pm0.34$	$5.23^{c}\pm0.39$	$4.87^{d}\pm0.25$
w(reducing sugar as glucose)/%	$3.49^{a}\pm0.14$	$2.54^{b}\pm0.25$	$3.47^{b}\pm0.79$	$1.87^{c}\pm0.06$	$0.59^{d}\pm0.14$	$0.21^{e}\pm0.08$
w(total protein $N_2\times6.25$)/%	$18.46^{a}\pm0.04$	$20.84^{b}\pm0.15$	$21.76^{b}\pm0.02$	$21.97^{c}\pm0.03$	$21.31^{c}\pm0.61$	$23.29^{d}\pm0.45$
w(soluble protein)/%	$54.01^{a}\pm0.90$	$52.98^{a}\pm2.02$	$56.16^{a}\pm3.67$	$62.63^{b}\pm1.82$	$65.60^{b}\pm1.69$	$59.17^{d}\pm1.71$
w(amino nitrogen)/(mg/100 g)	$6.94^{a}\pm0.89$	$6.64^{a}\pm0.01$	$6.08^{a}\pm1.42$	$8.39^{b}\pm0.02$	$8.50^{b}\pm0.37$	$6.11^{c}\pm0.02$
w(total ash)/%	$4.44^{a}\pm0.03$	$4.81^{b}\pm0.24$	$4.81^{b}\pm0.07$	$5.09^{c}\pm0.11$	$5.75^{d}\pm0.24$	$5.51^{d}\pm0.39$

Results are the mean of triplicates±S.D. and on dry basis
*indicates the appearance of surface mould on the product
Means marked with different letters (a, b, c, d, e, f) in the same row indicate the significant ($p<0.05$) difference

Table 5. Biochemical changes during the fermentation of douchi at 40 °C

Parameters	Dough	0 h	12 h	24 h	48 h*	72 h*
Acidity/%	$0.83^{a}\pm0.06$	$1.09^{b}\pm0.05$	$2.22^{c}\pm0.11$	$2.47^{d}\pm0.09$	$1.49^{e}\pm0.13$	$0.29^{f}\pm0.07$
pH	$6.23^{a}\pm0.02$	$6.04^{b}\pm0.02$	$5.01^{c}\pm0.03$	$4.93^{d}\pm0.04$	$5.67^{e}\pm0.05$	$6.27^{f}\pm0.05$
w(free sugar as glucose)/%	$9.19^{a}\pm0159$	$8.54^{a}\pm0.39$	$7.74^{b}\pm0.18$	$6.91^{c}\pm0.28$	$6.73^{c}\pm0.59$	$5.04^{d}\pm0.19$
w(reducing sugar as glucose)/%	$4.96^{a}\pm1.08$	$4.57^{a}\pm0.16$	$4.58^{a}\pm0.18$	$2.95^{b}\pm0.32$	$0.73^{c}\pm0.05$	$0.26^{d}\pm0.09$
w(total protein $N_2\times6.25$)/%	$18.61^{a}\pm0.03$	$20.61^{b}\pm0.15$	$22.03^{c}\pm0.07$	$22.07^{c}\pm0.15$	$22.89^{d}\pm0.06$	$22.38^{e}\pm0.31$
w(soluble protein)/%	$49.01^{a}\pm1.45$	$52.59^{b}\pm0.87$	$50.70^{b}\pm2.77$	$55.32^{b}\pm4.67$	$53.04^{b}\pm0.76$	$55.36^{c}\pm0.66$
w(amino nitrogen)/(mg/100 g)	$6.33^{a}\pm0.71$	$7.35^{b}\pm0.02$	$7.77^{b}\pm0.32$	$8.76^{c}\pm0.32$	$7.68^{d}\pm0.03$	$7.14^{e}\pm0.46$
w(total ash)/%	$4.52^{a}\pm0.10$	$4.89^{b}\pm0.11$	$5.04^{b}\pm0.17$	$5.45^{c}\pm0.17$	$5.76^{c}\pm0.17$	$5.78^{c}\pm0.08$

Results are the mean of triplicates±S.D. and on dry basis
*indicates the appearance of surface mould on the product
Means marked with different letters (a, b, c, d, e, f) in the same row indicate the significant ($p<0.05$) difference

pH values of about 4.74 were achieved at 30 °C, while at the same temperature, after 24 h of fermentation acidity was about 2.39 % and pH=4.83.

The biochemical changes observed (Tables 3–5) are probably the result of the microbial activities, especially of lactic acid bacteria, in the dough involving fermentation, which increased the acidity and reduced the pH values at the initial stages of fermentation. The rise in the level of soluble constituents, including soluble protein and amino nitrogen, is presumably due to the production of the proteolytic enzymes by the growth of developing microorganisms and the enzymatic hydrolysis of insoluble polymers under the acidic conditions. Increase in the acidity of the products helps in enhancing the shelf life of the food. Increase in the acidity, reduced pH, increase in soluble and free amino acids, B vitamins, increased solubility and increased digestibility have also been reported during the preparation of other traditional foods like Indian wari, tempeh, gari and miso (*11,12*).

The decrease in the acidity levels and increase in the pH at the later stages of fermentation at 30 and 40 °C were also observed. Mould on the surface of douchi products was also observed in the later stages of fermentation. These products are considered to be of lower quality. The decrease in the acidity in the later stages of fermentation is presumably due to the utilization of protein by the mould. Similar decrease in the pH from 5.5 to 4.1 at the initial stages of fermentation after 24 h but increase to pH=5.5 after 48 h was also observed during the mungbean tempeh fermentation (*12*).

Sensory evaluation of the douchi samples was carried out. The mean panel score of smell, taste, flavour and overall acceptability following two-way ANOVA for the factorial design with replication followed by mean separation technique between the time factor and the temperature factor is given in Table 6. Significant ($p<0.05$) difference within the scores among time, temperature and significant ($p<0.05$) interaction between time and temperature was observed.

The results indicate that fermentation conditions of 30 °C for 24 h and 30 °C for 48 h were significantly ($p<0.05$) superior among other fermentation conditions in terms of smell, taste, flavour and overall acceptability. The sensory score of douchi fermented for more than 48 h at 30 °C and for more than 24 h at 40 °C was lower, which means it was of poor quality.

The selection of optimum fermentation temperature and time are the major factors affecting the sensory quality of the products. Lower pH and higher acidity produced superior organoleptic results of douchi. The pH value of about 4.5 is also safe against most of the pathogenic microorganisms, which makes this product of superior quality. Optimum fermentation also results in the nutritionally superior product. Increase in the protein and starch digestibility as well as biological values have also been reported during the fermentation (*11,13*).

Table 6. Mean panel score for smell, taste, flavour and overall acceptability of douchi prepared under different fermentation conditions

Processing steps	20 °C	30 °C	40 °C	Remarks
t/h		Smell		
12	2.6^{f}	2.4^{j}	3.4^{k}	
24	2.4^{f}	$\mathbf{3.7^{m}}$	2.7^{j}	TM
48	3.5^{g}	3.5^{k}	1.8^{f}	$\mathbf{3.2^{p}}$
72	3.3^{g}	1.5^{f}	1.5^{f}	
		Taste		
12	2.7^{l}	2.3^{j}	3.2^{g}	
24	2.6^{f}	$\mathbf{3.8^{m}}$	2.9^{j}	TM
48	2.9^{i}	3.6^{h}	1.4^{f}	$\mathbf{3.1^{q}}$
72	3.1^{i}	1.5^{f}	1.3^{f}	
		Flavour		
12	2.7^{f}	2.7^{j}	3.9^{k}	
24	2.7^{f}	$\mathbf{4.2^{m}}$	3.0^{j}	TM
48	3.3^{i}	4.0^{h}	1.5^{f}	$\mathbf{3.5^{q}}$
72	3.2^{i}	1.7^{f}	1.6^{f}	
		Overall acceptability		
12	2.5^{f}	2.7^{j}	3.8^{k}	
24	2.7^{f}	$\mathbf{3.7^{m}}$	3.0^{j}	TM
48	3.6^{g}	3.6^{k}	1.9^{f}	$\mathbf{3.4^{q}}$
72	2.9^{i}	1.8^{f}	1.5^{f}	

TM indicates the traditional douchi prepared by sun drying
Means marked with different letters in the same column and row indicate the significant ($p<0.05$) difference
Means given in bold face with different letters (p, q) are significant ($p<0.05$) difference
Significant interaction was observed between the time and temperature

In conclusion, douchi prepared by controlled fermentation and drying is sensorially superior as compared to the traditional douchi prepared by sun drying. Controlled fermentation and mechanical drying method can be used to commercialize the production of douchi.

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Utjecaj temperature fermentacije i vremena na fizikalno-kemijske i senzorske značajke leguminoze Douchi

Sažetak

Proučeni su podaci o fizikalno-kemijskim i senzorskim značajkama leguminoze Douchi pripravljene fermentacijom pri različitim temperaturama prije mehaničkog sušenja. Fermentacije su se provodile pri 20, 30 i 40 °C tijekom 12, 24, 48 i 72 sata prije sušenja pri 50 °C. Preispitane su dobivene fizikalno-kemijske i senzorske karakteristike. Na osnovi dobivenih rezultata uočeno je da se fermentacijom pri 30 °C tijekom 24 i 48 h dobiva kiseliji Douchi (manje pH-vrijednosti) s boljim senzorskim značajkama.