ABSTRACT
The research focused on studying the impact of sourdough with spontaneous flora (SSF) (obtained also from gluten-free flour) and artisanal technologies on the quality indices of cozonac — a traditional pastry product. Physico-chemical and microbiological indices were analyzed, as well as glycemic index (GI) of cozonac samples with different fermentation agents. The experimental results showed that the GI of the cozonac samples have similar values, being between GI = 68 and GI = 71. Respectively, cozonac with SSF samples can be classified in the category of foods with moderate GI, and cozonac with commercial yeast (CY) — in the category of foods with high GI. Digestibility indices for all samples reached values between 72% and 76%. The sensory profiles of baked cozonacs were analyzed descriptively by the panel members, and the results showed that all cozonac samples were characterized by slightly acid taste, with specific and pleasant flavors. However, it seems that a long fermentation of the dough, even if CY is used as a fermenting agent, leads to the formation of quality indices of the dough and cozonac, very close to the products obtained only with SSF.

The use of SSF from sorghum flour would be an alternative in the development of gluten free bakery and pastry products using artisanal technologies.

KEY WORDS:
cozonac, sourdough, fermentation, artisanal technologies, soriz (Sorghum oryzoidum), nutritional aspects

1. Introduction
Technologies inspired by nature for a sustainable future! These are the trends of the modern consumer: quality products based on "bioinspired" technologies, obtained from natural and quality materials. Trends that are found in the first objectives of the 2030 Agenda for Sustainable Development are a challenge for a healthy future! Consumer concern about traditional artisanal products is on the rise [1]. This situation generates a demand for agricultural or food products with certain identifiable characteristics, in particular as regards their geographical origin. Currently, there is an upward trend of consumers to benefit from products not only with optimal organoleptic properties, but also with increased nutritional values.

Symbol of the fundamental holidays of Christianity, the cozonac also has the symbolism of bread: bread, in its Eucharistic form, refers to active life. And the active principle of fermentation becomes a symbol of spiritual transformation. Cozonac is a sweet bread, traditional in the Republic of Moldova, Romania, Bulgaria (kozunak), being traditionally cooked for Easter or Christmas. A similar sweet is "panettone" in Italy, in Russia "kulich", in Ukraine and Belarus "paska". According to some sources, the cake in the form and recipe known in the Republic of Moldova and Romania by all of us, is a Romanian invention.

The Bulgarian researcher, ethnologist Donka Sabotinova, says that he arrived in Bulgaria around 1915, brought from Romania by merchants around the Easter holidays, traded under the name of kozunak. It seems that the name cozonac comes from the Greek — κοσωνάϰι (kosonáki).

Cozonac in its simplest form is a sugar-sweetened flour mixed with eggs, milk, fat and a fermenting agent, but for every country and region there is a huge variety of recipes.

Currently, sourdough with spontaneous flora (SSF) is used more and more frequently in the production of artisanal products such as: panettone, pandoro, cozonac, typical regional bakery products, bringing rheological, sensory and nutritional benefits, as well as extending the shelf-life of the product. Sourdough is seen as an intermediate transition between the mixture and the final product, in which the active metabolic microorganisms modify the original characters of the initial ingredients (water and flour), a process that stimulates the interests of researchers [2,3].

There are multiple methods of making SSF. In general, this involves a mixture of water and flour and possibly salt and sugar, which is left to ferment for about 24 hours. At this stage the yeasts and lactic bacteria, naturally present in the flour, produce CO2 and organic acids. Reducing the pH activates the flour proteases, which, together with the hydrolytic enzymes of the bacteria, act on gluten, leading to a reduction in the consistency (fluidization) of the mixture. In the second stage, the mixture is refreshed to ensure oxygenation and provide a new nutrient substrate for microorganisms. The refreshments are repeated at certain intervals, until the fermentation capacity is kept constant. It is considered that the optimal time to make the refresh is determined by increasing the volume of the mixture about 3–4 times compared to the initial volume [4].

Regardless of the procedure involved, the microbiological composition of the SSF is represented by a mixture of lactic bacteria and yeasts in a ratio of 100:1, with respective values of 109 and 107 CFU/g [4,5].

It is considered that the use of SSF in the manufacture of bakery and pastry products has many advantages:

- improving the rheological properties of the dough (by accumulating metabolites, respectively amino acids);
- obtaining products with a better flavor and texture compared to products fermented only with commercial yeast;
- improving the nutritional values of products by increasing the bioavailability of minerals and reducing the glycemic index;
- increasing the shelf life of the products, through the inhibitory effect on molds possessed by organic acids, formed during fermentation;
- low pH inhibits amylase activity, so that starch degradation is avoided;
- fermentation with SSF improves water binding capacity, starch swelling and solubility of pentosans etc.
The aim of the research is to study the impact of artisanal technologies (the use of SSF and the long fermentation time) on the quality indices of cozonac — a traditional product of the Moldovan people.

2. Materials and methods

The quality of finished products depends on several factors, among which the most important are: the quality of the raw material and the technological process. For the preparation of SSF, as well as cozonacs, 2 types of local flour were used:

- high quality wheat flour (origin: Măgdăcești village)
- sorghum flour (Sorghum Oryzoidum) (SC „Andigor”).

The quality indices of the flours used are presented in Table 1.

| Quality indices                  | Wheat flour | Soriz flour |
|----------------------------------|-------------|-------------|
| Acidity, (degrees)               | 2.8±0.2     | 3.2±0.3     |
| Dry substance, %                 | 13.45±0.3   | 12.7±0.2    |
| Wet gluten, (%)                  | 26.18±0.7   | —           |
| Dry gluten, (%)                  | 61.0±1.5    | —           |
| Hydration capacity, (%)          | 51.0±0.5    | 100.0±1.0   |
| Maltose index, g / 100 g         | 0.5±0.2     | 2.3±0.1     |

The research was performed on the following cake samples:

- Cozonac of wheat flour with commercial yeast (CY) — standard sample;
- Cozonac of wheat flour with sourdough with spontaneous flora (SSF) of wheat flour;
- Cozonac of wheat flour with sourdough with spontaneous flora (SSF) of soriz flour.

SSF were obtained in laboratory conditions, by mixing wheat / soriz flour with water in proportions of 50/50. The mixture obtained, being subjected to fermentation under the influence of spontaneous flowering for 66 hours with periodic refreshments (Figure 1).

In order to characterize and evaluate the quality of cozonacs with SSF, the parameters mentioned in Table 2 were identified.

### Table 1

| Quality indices                  | Wheat flour | Soriz flour |
|----------------------------------|-------------|-------------|
| Loss of mass at baking           | GOST 21094–75 [7] |
| Moisture                         | GOST 21094–75 [9] |
| pH                               | 26-PH-metr-WTW-Inolab-ph-7110 |
| Porosity and elasticity of the crumb | GOST 5669–96 [10] |
| Microbiological indices (the total number of yeasts and molds) | GOST 10444.1 2–88 [11] |
| Glycemic index (in vivo method)   | ISO 26642–2010 [12] |
| Protein digestibility in vitro   | Sudeash, J. et al. [13] |
| Sensory indices                  | GOST ISO 6658–2016 [14] |

The glycemic index of the tested samples was determined in vivo by monitoring the blood glucose level of the experiment participants until and after the consumption of the researched food products, according to ISO 26642: 2010 [12]. The glycemic response after consumption of each product was compared with that stimulated by glucose consumption as a reference substance [15]. The data obtained were used to construct the glycemic response curves of the participants after consuming the tested samples. The area of the surface under curves was determined by mathematical method with the help of AutoCAD through the program “Inquiry” which calculates exactly the area of the surface. Finally, the glycemic index was calculated according to the formula:

\[
GI = \frac{Sa}{Sg} \times 100; \tag{1}
\]

where:

- \(GI\) — glycemic index of the analyzed food;
- \(Sa\) — the surface area under the glycemic curve of the studied food;
- \(Sg\) — the surface area under the glucose curve of glucose.

The blood glucose in the capillary blood of the experiment subjects was determined by the endpoint glucose oxidase method at the biochemical analyzer “STAT-FAX 1904” [16]. Principle of the method: Glucose, under the action of glucosidase, is con-
verted into gluconic acid. The resulting H2O2 will be decomposed by peroxidase, following the reaction in which the Trinder indicator (phenol and 4 amino antipyrine) also participates, resulting in a red-colored condensation product with maximum absorption at $\lambda = 505$ nm. Extinction is directly proportional to glucose concentration [17].

3. Results and discussion

Cozonac, a typical traditional product, symbol of Christmas and Easter, once cooked only by artisanal technologies, now broadcast at industrial level, is characterized by a strong spongy core, consistency and flavor unmistakable. The cozonacs were obtained from the culinary technology laboratory of the Food and Nutrition Department, Technical University of Moldova, by the indirect method according to the technological scheme (Figure 2):

3.1. Physico-chemical indices

The average humidity values of the cozonac samples were about: 25% for the cozonac with CY, 26% for the cozonac with SSF from wheat flour and 23% for the cozonac with SSF from soriz flour. In the last stage of the technological process, the product undergoes physical / structural and biochemical changes, which are crucial for acquiring rheological, sensory and nutritional characteristics. The samples obtained, after baking, were cooled to $t = 18–20^\circ$C for 8 hours, to strengthen the structure of the product after which they were subjected to determinations [18].

The values of mass losses at baking were close to all samples, being between 10–13% (Figure 3). The smallest being in the samples of soriz flour, probably due to the specific feature of agglutinative flours to retain a larger amount of water. The loss of mass results from the loss of moisture, from the outer layers of the dough. In the literature these losses are between 6–22% [4, 19].
The porosity of the investigated cozonac samples was between 85% (for the wheat SSF cozonac) and 79% (for the sorghum SSF cozonac) (Figure 4). The porosity of the SSF cozonac sample from wheat flour is 1% higher than the standard sample (CY cozonac) (Figure 4).

The elasticity was between 76÷96%, the most elastic being the cozonac with SSF from wheat (96%), and the least elasticity was the cozonac with SSF from soriz (76%), probably due to the gluten free properties of the sorghum flour from which the SSF was obtained. However, the differences between values, for all cozonac samples, are not considerable and could be explained by the formation of acids in the fermentation process, and as a result there is swelling and development of gluten proteins, which potentially contributes to a more efficient intercalation within the elastic network (Figure 5).

The pH was 4.46 in the CY cozonac, in the wheat flour SSF cozonac- pH = 4.52 and pH = 4.54, respectively, in the sorghum SSF cozonac and is due to organic acids, in particular lactic acid formed in the fermentation process of SSF and subsequently of the dough.

The acidity values for all samples are close, because even in doughs fermented with commercial yeast, the acidification is due primarily to the lactic acid produced by the lactic microflora when the fermentation exceeds 8–12 hours and, secondly, to the production of succinic acid by Saccharomyces cerevisiae [20,21,22]. The pH of the cozonac samples decreased slowly during the storage period (30 days) to 4.40, 4.49 and 4.52, respectively. The results obtained are close to the values for artisanal Italian panettone whose pH initially had values of 4.54 and decreased to 4.12 after 180 days of storage [19].

3.2. Microbiological indices

To determine the total number of yeasts and molds, inoculation was performed on Yeast Extract Glucose Chloramphenicol Agar (YGCA), followed by incubation under aerobic conditions at 25oC for 5 days. The results are shown in Table 3 and are characteristic for artisanal bakery products with SSF [19].

Table 3

| Cozonac samples                  | the total number of yeasts and molds, CFU |
|----------------------------------|-----------------------------------------|
| 1. cozonac with commercial yeast | 0.78±0.8                                 |
| 2. cozonac with SSF of wheat flour | 0.79±1.0                                 |
| 3. cozonac with SSF of soriz flour | 0.78±0.9                                 |

3.3. Nutritional indices

In vitro protein digestibility. Heat treatment beyond 95°C is considered to have both a beneficial and inhibitory influence on

![Figure 3. Images of the process of obtaining cozonacs](image3.png)

![Figure 4. The impact of SSF on the elasticity and porosity of cozonacs](image4.png)

![Figure 5. Baking mass losses](image5.png)
protein digestibility. Beneficial effects include inactivation of digestive enzyme inhibitors and development of heat-induced protein structure, while aggregation of denatured proteins resulting from new molecular interactions (little or no attack of digestive enzymes) and have the opposite effect. It is also considered that a major impact on digestibility has a microstructure of gels, formed by proteins, starch, etc. which affects the diffusion of enzymes in the food system and therefore the enzymatic degradation of proteins [23,24]. The digestibility values (with trypsin) of the cozonac samples reached values between 72 and 76% (Table 4).

**Table 4**

| Cozonac samples               | Digestibility, % |
|-------------------------------|------------------|
| 1. cozonac with commercial yeast | 72 ± 1.0         |
| 2. cozonac with SSF of wheat flour | 76 ± 1.0         |
| 3. cozonac with SSF of soriz flour | 75 ± 1.6         |

The values of the digestibility indices of the cozonacs could be explained by the fact that all the samples (although they had different fermentation agents), took place in identical conditions of time and environment. In the literature there are indices of protein digestibility in bakery products, with values between 79.96 and 80.62 [25].

**Glycemic index.** The average pre-prandial glycemia of participants in the experiment was in the optimal range of 3.8 ± 0.8 mmol/l. After consuming the samples examined maximum glycemia was reached over 30 minutes.

The experimental results showed that the GI of the cozonac samples have similar values, being between GI = 68 and GI = 71 (Figure 6). Although GI values do not differ significantly, cozonacs with SSF can be included in the category of foods with moderate GI, and cozonacs with CY — in the category of foods with high GI [15,26,27,28]. However, experiments on GI are required, given the multitude of factors that can influence this index. According to the literature, the values obtained are characteristic of bakery and pastry products: cupcake — GI = 73 ± 12, croissant-GI = 67, bread au lait — GI = 63 ± 10, baguette=GI = 95 ± 15 [26].

### 3.4. Sensory indices

As mentioned in the literature, any change in the technological process of obtaining cozonacs or in the development of the recipe can lead to changes in their quality. The volatile compounds in the kernel derive, in particular, from the fermentation process of SSF, from the oxidation of lipids in flour and, to a lesser extent, from the Maillard reaction, while the aroma of the crust is mainly due to the Maillard reaction. The sensory profiles of baked cozonacs were analyzed descriptively by the panel members (specialists from the Department of Food and Nutrition, Technical University of Moldova), assessing the intensity of each (Table 6) and a hedonic parameter — as a general assessment [29,30].

**Table 5**

| Sample                                      | 0     | 15    | 30    | 45    | 60    | 90    | 120   | 180   |
|---------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Glycemia, mmol/l                            |       |       |       |       |       |       |       |       |
| glucose                                     | 3.8±0.8 | 5.6±1.1 | 6.8±1.2 | 5.6±0.9 | 4.5±0.7 | 4.9±0.9 | 4.4±0.6 | 3.7±0.4 |
| cozonac with CY                             | 3.8±0.7 | 4.8±0.8 | 6.1±0.8 | 5.2±0.2 | 4.5±0.4 | 4.5±0.4 | 4.2±0.6 | 4.1±0.6 |
| cozonac with SSF of wheat flour             | 3.8±0.6 | 4.8±0.6 | 5.9±0.9 | 5.0±0.4 | 4.6±0.4 | 4.4±0.4 | 4.1±0.6 | 4.1±0.6 |
| cozonac with SSF of soriz flour             | 3.8±0.6 | 4.9±0.8 | 5.8±0.9 | 5.5±1.0 | 4.5±0.5 | 4.2±0.5 | 4.1±0.5 | 4.0±0.5 |

**Table 6**

| Sensorial evaluation of cozonac crumb         | Aspect                 | Smell      | Taste     | Structure |
|-----------------------------------------------|------------------------|------------|-----------|-----------|
|                                               | Intensity of color     | Cereals    | Sweet     | Elasticity|
|                                               | Luminosity             | Acetic acid| Acid      | Deformability|
|                                               | Density                | Hay        | Bitter    | Resistance to chewing|
|                                               | Porosity               | Yeast      | Cereals Flavor | Surface moistness|
|                                               | Homogeneity            | Rancid     | Hay Flavor | Compactness|
|                                               |                        |            | Yeast flavor | Cohesiveness|
|                                               |                        |            | Astringent | Juiciness|
|                                               |                        |            |            | Aftertaste|

| Sensorial evaluation of cozonac crust         | Aspect                        | Smell          | Taste    | Structure                                      |
|-----------------------------------------------|-------------------------------|----------------|---------|-----------------------------------------------|
|                                               | Intensity of colour           | Intensity of smell | Sweet | Structure regularity |
|                                               | Regularity of colour          | Cereals        | Acid | Hardness |
|                                               | Tonality of colour (yellow/brown) | Fragrant | Bitter | Friability |
|                                               |                               | Roasted        | Hay flavor | Crispness |
|                                               |                               | Burned         | Yeast flavor | Resistance to detachment (crust/crumble) |
|                                               |                               |                | Astringent |                   |
|                                               |                               |                | Aftertaste |                   |
The cozonacs obtained with different fermentation agents (CY, SSF with wheat flour, SSF with sorghum flour) were characterized by close sensory profiles. Cozonacs with SSF from sorghum flour had the weakest sensory profile in terms of structural characteristics, but the best aromatic profile. The wheat flour SSF cozonac showed optimal structural characteristics of the core and crust, and the CY cozonac, despite a good consistency, had an uneven crust both in color and structure. All cozonac samples were characterized by slightly acid taste, with specific, pleasant flavors.

4. Conclusions

- The tendencies of specialists in the field of producing with artisanal technologies are based on the use of local traditional raw materials, which give the products specific texture and sensory characteristics;
- The artisanal processes in the elaboration of cozonacs do not necessarily involve high quality flours, but they require increased amounts of fluids (water, milk etc.) and a long kneading (about 40 minutes) for the formation of the gluten network;
- SSF is the key element in the development of artisanal products due to its ability to improve flavor and potential nutritional aspects;
- Fermentation is the fundamental process in obtaining cozonacs with SSF and is also characterized by specific time and temperature parameters;
- The parameters of the technological process, such as time, temperature and consistency influence the characteristics of the dough and, as a result, of the cozonac;
- The final product has a slightly acidic flavor, obtaining particular organoleptic properties, due to the production of metabolites (products of the metabolism of many microbial species present in SSF), which gives a more complete and richer aroma, but also a longer shelf life compared to industrial products;
- However, it seems that a long fermentation of the dough, even if CY is used as a fermenting agent, leads to the formation of quality indices of the dough and cozonac, very close to the products obtained only with SSF;
- The use of SSF from sorghum flour would be an alternative in the development of gluten free bakery and pastry products using artisanal technologies.

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The authors declare no conflict of interest.

Received 12.08.2020 Accepted in revised 03.09.2020 Accepted for publication 25.09.2020