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The Potential of Georgian Ojaleshi Grape Juice for the Production of fermentation Functional Beverages

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Abstract

This study explores the potential of the Georgian Ojaleshi grape variety for producing non-alcoholic fermented beverages, offering an alternative for those with lactose intolerance, milk allergies, or following a vegan diet.
After 8 days of fermentation, the juice showed reduced sugar levels, increased phenolic compounds, and enhanced antioxidant activity. Sensory analysis revealed a sweet-sour taste, refreshing flavor, slight effervescence, and a pleasant aroma. The alcohol content was 0.42%, making it a non-alcoholic drink with potential health benefits.

Keywords

Grape, Fermentation, Functional beverage, Total sugars, Probiotic.

Introduction

In recent years, profound and significant changes have been observed in human consciousness regarding the physiology of nutrition. Nutrition and health are oriented towards a new strategy - the production of functional products. The role of fruits in this regard is indisputable - there is consistent and solid evidence in this regard [1,2,3,4,5,6] phytonutrients have a certain physiological role [EC 1924/2006; EU 2012/12], are characterised by high antioxidant activity, and have a preventive effect against chronic diseases [7].
Noncommunicable diseases are a leading cause of morbidity and mortality, recognised as a significant challenge in achieving the UN Sustainable Development Goals "Time to Act: Accelerating our response to NCDs for the health and well-being of current and future generations" [UN/2018].
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Over the past decades, the nature of nutrition has changed for the worse.
Today's food products are absolutely inadequate; they cannot meet the needs of the human body, which disrupts the balance

of chemical components formed in the body during the process of evolution. This leads to various diseases. The body tries to solve this problem on its own, but it needs help from the outside, which is what functional food can do.

The functional food market is developing due to the growing consumer interest in a healthy lifestyle. Fermented functional products, including beverages based on plant matrices, deserve special attention [8, 9]; the reason is such widespread problems as lactose intolerance, allergic reactions to milk proteins, high cholesterol, as well as vegan diets, etc. Fermented products have a positive growth trend in the market. It is estimated that by 2025, sales will reach approximately 26 billion US dollars [10].

Therefore, it is important to develop alternative plant matrices, innovative formulas, and technologies in this direction.

According to the available information, grapes have the potential to maintain human health, actually limit the development of chronic diseases; they are distinguished by a unique combination of phenolic compounds, therefore they have strong antioxidant activity and anti-microbial properties. It is believed that grapes and products derived from them should be included in the daily diet. The main thing is to use this opportunity correctly [11,12, 13].

It is important to consider the recommendations of the World Health Organisation (WHO, 2003) and the requirements of the European Regulation (EC 1924/2006), according to which it is necessary to significantly limit sugar consumption (>50 negative events have been identified).

The level of free sugar consumption in Georgia significantly exceeds the norms specified by the WHO recommendation [Guideline,2015/WHO] https://www.who.int/publications/i/item/9789241549028, which, together with the consumption of low-quality food, determines the growing trend of morbidity and mortality.

According to the latest data, at least 3/4 of the total morbidity and 94% of mortality in Georgia are due to diseases associated with nutrition.

Scientific studies have documented the role and benefits of prebiotics and probiotics for such a key system of the human body as the gastrointestinal tract. [14].

Modern requirements require a minimisation of sugars in soft drinks. Grape juice is high in calories ≥70 kcal / 100 g, which is mainly due to sugars.

Scientific studies have confirmed the negative impact of glucose on the human body. It is important to convert it into substances with different tastes and at the same time positive effects, such as: carotenoids, flavonoids, L-carnitine, choline, coenzymes, phytosterols, phytoestrogens, glucosinolates, polyphenols, taurine, amino acids, organic acids, etc.

These biochemical transformations will contribute to a significant reduction in glucose and a minimum of total sugars in soft drinks based on grape juice. This is achieved by non-alcoholic (or low-alcohol) fermentation of natural yeasts and/or bacteria and/or their synergism (similar action is exerted by milk mushrooms, kombucha tea, etc.), which determines the functional properties of the drink [15].

Thus, the study aims to assess the potential of using Georgian, in particular, the Ojaleshi grape variety for the production of non-alcoholic fermented drinks.

Materials and Methods

Grapes were chosen as the basic object of the study. They are a healthy food product, have a preventive effect on chronic diseases; their use needs to be optimized - they are characterized by a unique combination of phenolic compounds and strong antioxidant activity, the protective function is mainly determined by the data of the seeds and skin: hydroxycinnamic acids, hydroxybenzoic acids, resveratrol, flavan-3-ols, pro anthocyanidins; the skin of red grapes also contains anthocyanins.

The value of grapes is also determined by the pulp–hydroxycinnamic acids, flavan-3-ols, anthocyanins (in some varieties); it has cardioprotective and anti-inflammatory effects [16], and the content of fiber also deserves attention.

The study was conducted on the Georgian grape variety Ojaleshi, which is an ancient Megrelian standard red grape wine variety; it is most common in Samegrelo, Imereti and Racha-Lechkhumi.

Ojaleshi grape berries are dark blue in color, ripen in late November-early December, accumulate up to 20-23% sugar, and the acidity is 0.8÷0.9%; the pulp is fleshy, with a pleasant sweet taste and rich varietal aroma.

It is characterized by a high quantitative and qualitative content of antioxidant compounds [17,18,19].

The research aims to study the fermentation process of Ojaleshi juice and assess the potential of using Ojaleshi grapes to produce non-dairy probiotic beverages.

Fermentative fermentation is mainly carried out by symbiotic cultures of bacteria (Komaga-taeibacter,Acetobacter, Glucono-bacter) and yeasts (Zygosaccharomyces spp., Saccharomyces spp. and Brettanomyces spp.) [20,21]. The participation of lactic acid bacteria (Lactobacillus, Leuconostoc) has also been recorded [22, 23].

Fig.1. Ojaleshi

Yeast hydrolyzes sucrose into glucose and fructose - by-products of ethanol fermentation, while acetic acid bacteria convert ethanol into acetic acid and gluconic acid [24,25], and other metabolites are also formed: lactic acid, citric, malic and glucuronic acids, vitamins, minerals, phenolic compounds, etc. [26, 27].

The fermentation of the Ojaleshi juice was carried out with a synergistic combination of microorganisms, which included yeasts isolated from the kvass (Zygosaccharomyces bailli and Saccharomyces cerevisiae), lactic acid and acetic acid bacteria (Acetobacter xylinus, Acetobacter pasterianus).

The experiment was carried out in the following sequence: we filled a three-liter glass vessel with Ojaleshi juice to a volume of 2 liters. We added lactic acid and acetic acid bacteria, yeasts from the collection of microorganisms to the solution, then covered it tightly with a thick layer of gauze (5-6 layers). Incubation was carried out in a thermostat for 10 days at a constant temperature of 20 °C.

The main physicochemical and phytochemical parameters important to us were determined in the analytical samples taken every 24 hours after inoculation of the Ojaleshi base juice with microorganisms: soluble dry matter - by refractometric method, pH active acidity - by pH-meter (ISO 1842:1991 Fruit and vegetable products - Determination of pH) [28], total sugars - by the DNA method [Miller et al. 1959] [29], total phenolic compounds – by Folin-Ciocalteu reagent with GAE standards [30], ethyl alcohol - by the FTIR (Fourier transformed infrared spectroscopy) method, as well as - organoleptic parameters using the hedonic scale [31]. It should be noted that for greater accuracy, the above experiments were carried out with 3-fold repeatability. The statistical processing method of analysis ANOVA is used for data processing.

Results

- At the initial stage, the chemical and phytochemical parameters of Ojaleshi juice were determined, which are given in Table 1. The chemical and phytochemical parameters given below were determined in the fermented juice samples taken every 24 hours for 10 days. After inoculation with Ojaleshi juice microorganisms.
- **Change of pH** - Table 2 shows the hourly active acidity indicators of the juice from the start of fermentation.
- **Change of total sugars** - Table 3 shows the hourly total sugar indicators of the juice from the start of fermentation.
- **Change of total phenolic compounds** - Table 4 shows the hourly total phenolic indicators of the juice from the start of fermentation.
- **Determination of ethyl alcohol in initial and fermented Ojaleshi juices** - Table 5 shows the hourly Ethyl alcohol indicators of the juice from the start of fermentation.
- **Sensory analysis** - Table 6 and Fig. 5 show the results and the diagram of organoleptic analysis of the fermented Ojaleshi juice. The fermented juice of Ojaleshi was rated by the consumer on a 5-point hedonic scale: 1 - very bad, 2 - bad, 3 - satisfactory, 4 - good, 5 - very good.

Discussion

The dynamics of pH changes during the fermentation process of Ojaleshi juice were determined; the pH of the control juice (natural Ojaleshi juice) was 3.4; 24 hours after the start of fermentation, a decrease in the pH of the juice was noted (pH 3.2); and after 10 days, the pH was 2; The quantitative change in total sugars during the 10-day fermentation period of Ojaleshi juice was determined.

The total sugars in the unfermented juice were 19 g/100 ml. During the fermentation process, the total sugars decreased significantly and amounted to 12.5 g/100 ml in the 10-day fermented juice.

The total phenolic content was determined in the initial and fermented juice samples of Ojaleshi.

The highest value was observed on the 8th day of fermentation (3,520 mg/l), after which the total phenolic content decreased.

Our research aimed to determine the potential of Ojaleshi juice for producing a non-alcoholic fermented beverage.

Naturally, it became necessary to determine the alcohol content in both control and fermented juice samples and to perform a comparative analysis of the data obtained.

Natural Ojaleshi juice was taken as a control, and the fermented juice on the 10th day was taken as the final sample. Alcohol in the samples was determined by infrared spectroscopy.

The results are given in The ethyl alcohol content was determined during the 10-day fermentation period of the Ojaleshi juice. During the fermentation process, the alcohol content increased slightly, the highest indicator was recorded on the tenth day - 0.48% (vol), which is less than 0.5% (vol) and corresponds to the indicator specified in the standard for non-alcoholic beverages.

It should also be noted that the characteristics of the eight-day fermented Ojaleshi juice turned out to be the most optimal in various respects, while the alcohol content was an acceptable figure - 0.42% (vol).

An important component of the experimental studies was the determination of sensory parameters of the research samples at different stages of fermentation and obtaining juice with acceptable (optimal)organoleptic characteristics.

The highest taste qualities were observed in the fermented juice on the 8th day; after that, acetic acid tones were noticeable in the juice. The fermented juice on the 8th day was pleasant to the taste, had the color and aroma characteristic of Ojaleshi juice, a slightly sweet-sour taste, and a tonic and refreshing effect.

Conclusion

Experimental studies have shown that during the 10-day fermentation period, under conditions of constant temperature of 20 oC, the total sugar content in the fermented juice on the 8th day decreased by 25%, which is a positive phenomenon and responds to the strict recommendations of international organizations on issues of nutritional physiology, in particular, regarding the consumption of free sugars; the quantitative content of total phenolic compounds increased by 42.7%, which is positively correlated with the antioxidant status of the juice.

It was determined that during the 10-day fermentation period, the best taste properties were revealed in the fermented juice on the 8th day, and the alcohol content was 0.42% (vol), which corresponds to the limits set by the standard for non-alcoholic beverages (≤0.5% (vol).

Thus, the changes in the Ojaleshi juice during the 10-day fermentation period were studied experimentally. According to the results of the studies, the optimal period of the fermentation process at a constant temperature of 20 oC is 192 hours (8 days and nights).

Ojaleshi grape juice is an interesting plant substrate for producing healthy, low-sugar, non-alcoholic probiotic drinks.

It should be noted that the research conducted in this direction is important because it contributes to expanding the spectrum of grape use and, in the future, the production of beverages that are relevant to health, of acceptable quality, with probiotic viability and organoleptic characteristics.

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Results Tables

1. At the initial stage, the chemical and phytochemical parameters of Ojaleshi juice were determined, which are given in **Table 1.**

dm, ∘B	Active acidity pH	Total sugars (%)	Total phenols, mg/l	Ethyl alcohol % (vol)
23.5	3.4	19	2466.7	0.1

Table 1. *Chemical and phytochemical data of Ojaleshi juice*

The chemical and phytochemical parameters given below were determined in the fermented juice samples taken every 24 hours for 10 days after inoculation with Ojaleshi juice microorganisms:

2. **Change of pH**

Table 2 shows the hourly active acidity indicators of the juice from the start of fermentatio

pH	Fermentation time, h										
	0	24	48	72	96	120	144	168	192	216	240
	3.4 ±0.002	3.2 ±0.001	3.1 ±0.001	2.8 ±0.001	2.6 ±0.001	2.5 ±0.001	2.4 ±0.001	2.3 ±0.001	2.2 ±0.001	2.1 ±0.001	2 ±0.001

Table 2. *Change of pH of Ojaleshi juice in the process of fermentation*

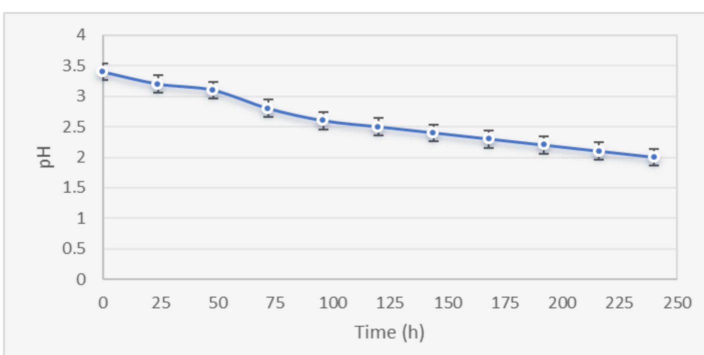

Fig. 1*. Change of pH of Ojaleshi juice during 10 days of fermentation*

3. **Change of total sugars** - Table 3 shows the hourly total sugars indicators of the juice from the start of fermentation.

Concentration of total sugars (g/100 ml)	Fermentation time, h										
	0	24	48	72	96	120	144	168	192	216	240
	19 ±0.3	18.5 ±0.2	18.1 ±0.2	17 ±0.1	16.5 ±0.1	16 ±0.1	15.8 ±0.1	15 ±0.15	14.3 ±0.15	13.5 ±0.1	12.5 ±0.1

Table 3*. Change of total sugars in the process of fermentation of Ojaleshi juice*

Based on the data obtained, a diagram of the quantitative change in total sugars was constructed, which is presented in **Fig. 2.**

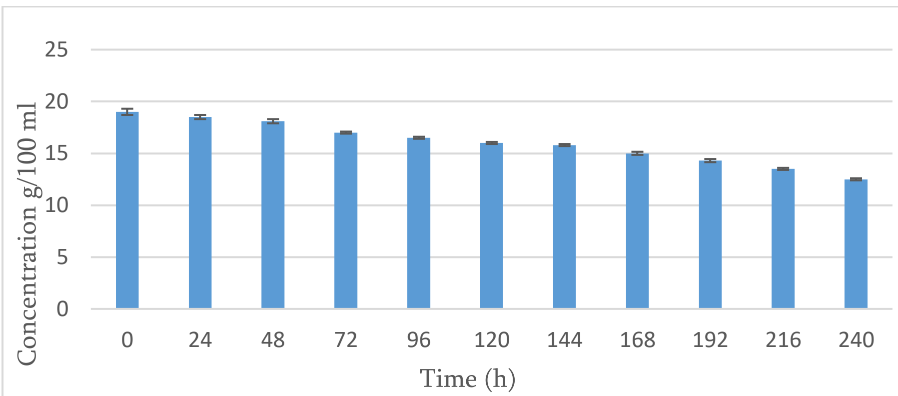

Fig. 2. Change of total sugars during the 10 days of fermentation

4. **Change of total phenolic compounds**

Table 4 shows the hourly total phenolic indicators of the juice from the start of fermentation.

Total phenol content in the test samples (mg/l)	Fermentation time, h										
	0	24	48	72	96	120	144	168	192	216	240
	2,466 ±0.05	2,576 ±0.045	2,596 ±0.04	2,866 ±0.03	2,900 ±0.03	2,950 ±0.035	3,100 ±0.03	3,360 ±0.02	3,520 ±0.02	3,470 ±0.015	2,872 ±0.02

Table 4*. Change of total phenols in the process of fermentation of Ojaleshi juice*

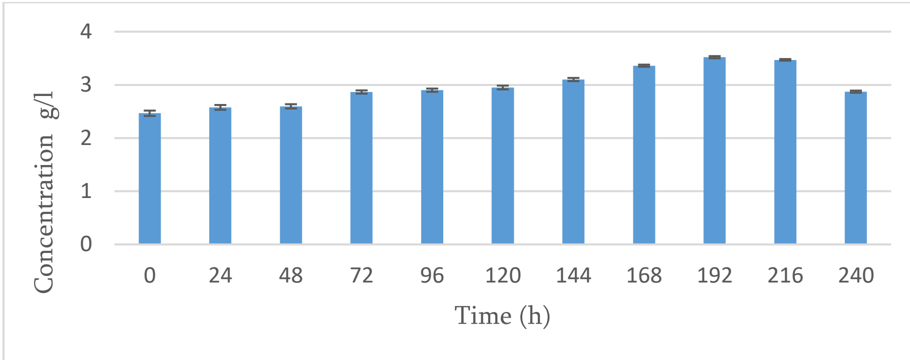

Fig. 3. *Change of total phenolic compounds during 10 days of fermentation*

5. **Determination of ethyl alcohol in initial and fermented Ojaleshi juices**

Table. 5 shows the hourly Ethyl alcohol indicators of the juice from the start of fermentation

Content of ethyl alcohol, % (vol)	Fermentation time, h										
	0	24	48	72	96	120	144	168	192	216	240
	0.1 ±0.002	0.14 ±0.001	0.17 ±0.002	0.2 ±0.001	0.25 ±0.001	0.3 ±0.001	0.36 ±0.001	0.39 ±0.001	0.42 ±0.001	0.46 ±0.001	0.48 ±0.001

Table 5. *Ethyl alcohol indicators in the samples*

According to the data, a diagram of the change in ethyl alcohol was constructed, which is presented in **Fig. 4**

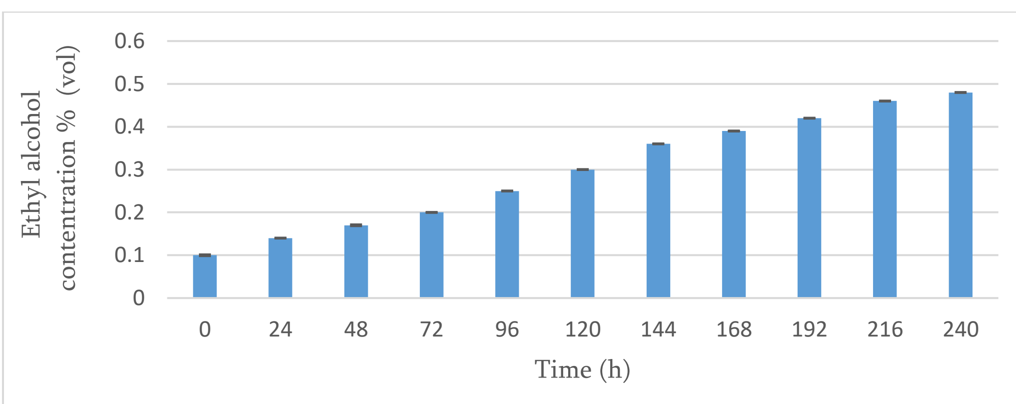

Fig. 4. *Change of ethyl alcohol during 10 days of fermentation*

6. **Sensory analysis -** Table 6 and Fig.5 shows the results and the diagram of organoleptic analysis of the fermented Ojaleshi juice. The fermented juice of Ojaleshi was rated by the consumer on a 5-point hedonic scale: 1 – very bad, 2 – bad, 3 - satisfactory, 4 - good, 5 - very good.

Hedonic scale	Very good	Good	Not good, not bad	Bad	Very bad
Color	5	5	0	0	0
Aroma	4	4	2	0	0
Taste	7	3	0	0	0
Odor	4	5	1	0	0

Table 6*. Results of organoleptic analysis of the fermented Ojaleshi juice*