

Scientific report

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Project: Innovative approach to develop value-added snack products through extrusion technology

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Project website: <https://fia.usv.ro/cercetare/inadext>

The research project aims to make better valorization of grape pomace obtained from some of the Romanian grape intensively cultivated varieties (white and red) in combination with maize flour by using extrusion technology to enhance snack production sustainability and to contribute to a lower ecological impact.

Relate to this concern, in the *second stage* of the project, assessed firstly the amino acid content of grape pomace and maize flour (A2.1) that wants to be used as raw materials in the extrusion process, activity according to the Annex II/Additional act 1/2022 of Contract no. PCE 60/2022. Data collection, elimination of aberrant results, and evaluation of the degree of validity and fidelity of the obtained results were performed.

Then, the feed mixtures for the extrusion process were prepared and their physicochemical characteristics, molecular characterization, and functional properties were determined (**O2** objective). The activities related to this objective (**O2**) from the second stage of the project 1/01/2023 - 31/12/2023, according to Annex II/Additional act 1/2022 of Contract no. PCE 60 / 2022, were as follows:

A2.1. Mixture preparation for extrusion, physicochemical characterization, and functional properties. Grape pomace will be mixed thoroughly with maize flour to the ratio of 0, 10, 20, 30, and 40% (w/w, d.b.), and the moisture, colour, ash, acidity, protein content, amino acids, fat, total dietary fiber, total phenolic content, antioxidant activity, degree of starch gelatinization will be determined. The following estimative results will be obtained: moisture, ash, acidity, colour, protein content, amino acids, fat, total dietary fiber, total phenolic content, antioxidant activity, starch gelatinization, and functional properties (water retention capacity and swelling capacity).

A2.2. Molecular analysis of grape pomace-maize flour mixtures formulated. A study of the molecular characteristics by using Fourier-transform infrared spectroscopy with attenuated transmission (FTIR-ATR) for the mixture formulated will be made.

A2.3. Data collection, elimination of aberrant results, and evaluation of the degree of validity and fidelity of the obtained results.

As deliverables associated with activities (A2.1, A2.2, and A2.3) specific to O2 objective, a database with physical-chemical, molecular characteristics, and functional properties of mixtures formulated for extrusion processing was achieved. Also, the scientific report on these characteristics of mixtures formulated for extrusion, which will be used in future experiments, was made.

Another objective (**O3**) includes testing, establishing the extrusion processing conditions of formulated mixtures, and performing extrusion. The activities related to this objective (**O3**)

from the second stage of the project 1/01/2023 - 31/12/2023, according to Annex II/Additional act 1/2022 of Contract no. PCE 60 / 2022, were as follows:

A3.1. Preliminary investigations for exploring and selecting the processing parameters and system parameters on mixtures formulated. Different parameter combinations of extrusion processing will be investigated by varying some parameters e.g., the levels of water added to the system, the feed rate, the temperature, the screw speeds, and specific mechanical energy. Due to the complexity, the parameters involved in the extrusion process will be tested to achieve the desired results, e.g., adequate expansion index, and density of the extrudates, by manipulating properties of feed mixtures and extrusion processing parameters. As deliverables associated with activity A3.1, a database with variable levels tested in different kinds of extrusion processing (e.g., feed moisture, grape pomace level in mixtures, feed rate, die temperature, die dimensions, screw speed, and specific mechanical energy) was achieved.

A3.2. Extrusion of formulated samples at establishing processing conditions, dried collected snacks extrudates and stored until analysis. The experimental design will be performed for grape pomace-maize flour mixture preparation. The independent variables will be e.g., die temperature, screw speed, and grape pomace level. The collected extrudates will be dried up to a final average moisture of 4-6% (w.b.) and stored in airtight plastic bags until analysis. As deliverables associated with activity A3.2, a database on variable levels established and used in the experimental design to obtain extrudates products was achieved.

A3.3. Data collection, elimination of aberrant results, and evaluation of the degree of validity and fidelity of the obtained results. As deliverables associated with activity A3.3 specific to the O3 objective, the scientific report contains variables levels tested in different kinds of extrusion process (e.g., feed moisture, grape pomace level in mixtures, processing temperature, and screw speed) established and used parameters in the experimental design to obtain desired products, was made.

The objectives of the second stage with specific activities and deliverables have been achieved.

Research materials and methods

The *research materials* and *methods* were presented in the following, to carry out the activities related to the objectives and to achieve the estimated results.

Maize flour and grape pomace represent the base materials used in experimental research. For these materials, amino acid content was determined in this stage. Additionally, feed mixture-based maize flour was prepared. For mixtures preparation (33 samples), maize flour (M) was partially substituted with 10, 20, 30, and 40% seedless grape pomace (SGP) or grape pomace with seeds (GP), from white (W) or red variety (R) variety, dried in a convective oven (O) or lyophilization (L).

The methods/standards used and the characteristics obtained are as follows:

- moisture determination / SR EN ISO 712:2010: moisture (%);
- determination of ash content by calcination / SR EN ISO 2171:2010: ash (%);
- acidity determination / SR 90:2007: acidity (degrees of acidity);
- colour parameters determination by using the colorimetric method: colour parameters in CIE Lab system: L^* , a^* , and b^* ;
- lipid content by using the Soxhlet method / AACC Method 30-25.01 or ICC 136: lipid (%);
- protein content by using the Kjeldahl method / AACC Method 46-12.01 or ICC 105/2: protein (%);
- amino acids determination by using HPLC technique (Shimadzu LC40-PDA-40 system), combined with pre-column derivatization of sample, in according with the Agilent - Amino Acid Analysis protocol: essential and unessential amino acids content (g/100 g)
- total dietary fiber content / AACC 32-05.01 method: total dietary fiber (%);
- differential scanning calorimetry (DSC): gelatinization onset temperature (T_o), peak temperature (T_p), final temperature (T_f), and the enthalpy (ΔH);

- total phenolic content (TPC) by using the Folin-Ciocalteu method: TPC (mg GAE/g dw);
- antioxidant activity determination by using 2,2 – diphenyl-1-picrylhydrazyl (DPPH) method: antioxidant activity (µg/mL);
- functional properties, water retention capacity, and swelling capacity: WRC (%), SC (mg/L);
- molecular characteristics by using Fourier-Transform Infrared (FTIR) spectroscopy: molecular characteristics: primary and secondary metabolites.

Results and discussions

The data collected from the research activities proposed and conducted during the second stage of the project, 1/01/2023 - 31/12/2022, according to Annex II/Additional act 1/2022 of Contract no. PCE 60 / 2022, was assessed from a statistical point of view, the aberrant results were eliminated, and then was evaluated the degree of validity and fidelity of the obtained data. Firstly, a database with amino acids content of grape pomace and maize flour was achieved. Then, for maize-based mixtures formulated with 10, 20, 30, and 40% whole grape pomace and seedless grape pomace, from white and red grape varieties, dehydrated by using a convective drying oven and lyophilization, respectively, a database with their physicochemical, molecular characteristics, and functional properties was achieved. This database with the results obtained will serve to evaluate the extrudability of the mixture formulated or as the basis for subsequent objectives, such as setting up the extrusion process parameters or conditions. As well, a database with variable levels tested in different kinds of extrusion processing, e.g., feed moisture, grape pomace level in mixtures, feed rate, screw speed, and die temperature, was achieved. Additionally, a database on variable levels established and used in the experimental design to obtain extrudates products was achieved.

The results obtained for *amino acids* content showed that the values were remarkable depending on the grape pomace type, seedless or with seeds. A high content of essential amino acids was found in whole grape pomace compared to seedless grape pomace, a fact which can be due to the essential amino acids present in seeds. There were no significant differences between grape pomace samples dried in an oven or through lyophilization. Compared to the essential amino acids present in maize flour, can be concluded that grape pomace represents an interesting source of economic amino acids to utilize in food preparation.

The physicochemical characteristics of mixtures formulated varied depending on the grape variety, the dried method, the type of grape pomace sample, seedless or with seeds, and the amount that substituted maize flour. An increase in *ash*, *lipids*, *protein*, and *fiber* content and a decrease of carbohydrates with grape pomace amount increase was observed in mixtures. The grape pomace features impacted the mixture's characteristics. There were no significant differences between mixtures formulated with the same amount of grape pomace dried in an oven or through lyophilization from the point of view of ash, lipids, and proteins. The mixtures with grape pomace presented up to 3 times more *lipids* and *fiber* compared to maize flour, the higher values being obtained for mixture with whole grape pomace compared to those with seedless grape pomace, especially from red grape variety. The lipids and fiber content rise with the increase of the amount of grape pomace in mixtures formulated. A high content of *proteins* was found in all mixtures with grape pomace from the red variety compared to those from the white variety. The *amino acids* content in the mixtures prepared was influenced by the amino acids present in studied grape pomace samples. There was no regular trend regarding amino acids content in mixtures with the increase of the amount of grape pomace which substitutes partial maize flour. An increase in essential amino acids was observed in mixtures including grape pomace with seeds compared to those with seedless grape pomace. Moreover, an enhanced profile in some *essential amino acids* such as: valine, methionine, phenylalanine, isoleucine, leucine, and lysine were obtained for mixtures compared to maize flour. The *acidity* values for all mixtures were within the recommended limits, even though there are variations from one type

of sample to another as a function of grape pomace type, grape variety, dried method, and the amount of grape pomace added in maize flour. The mixtures' *moisture* content is influenced by the grape pomace and maize flour moisture, but all mixtures formulated ensure suitable storage stability. The *colour* of the mixtures formulated for extrusion ranged with the amount of grape pomace added in maize flour and was impacted by the pigments from grape pomace and maize flour. Low values of luminosity (L^*) were observed in mixtures with whole grape pomace from the red variety compared with those with grape pomace from the white variety, and the lower L^* value was registered in the mixture with 40% grape pomace. With the increasing grape pomace content, the mixture's colour became darker, indicating that the grape pomace, seedless or with seeds, had a strong effect on the colour of the mixtures. The redness (a^*) parameter increased with the increased amount of grape pomace in mixtures, and higher values were obtained for the samples containing lyophilized seedless red grape pomace. An opposite trend was observed for the yellowness (b^*) parameter in the mixtures with lyophilized seedless red grape pomace. Measurements of *starch gelatinization* by differential scanning calorimetry revealed that the values for onset temperature (T_o), peak temperature (T_p), final temperature (T_f), and endothermic enthalpies (ΔH) for the gelatinization were different from each other. The grape pomace variety, sample type, seedless or with seeds, and the proportion that substituted maize flour impacted the thermal properties of the mixtures formulated. The possible interactions between grape pomace and maize flour constituents may modify the binding forces among the crystallites and amorphous network by linking to the amorphous area of starch grains and thus influenced gelatinization temperatures, T_o , T_p and T_f (Zhu et al., 2009). The increase of grape pomace amount in mixtures determined a decrease of ΔH for all mixtures. This effect can be related to the immobilization of water due to the high fiber content from mixtures formulated which also determine an increase in gelatinization temperatures. Regarding the *total phenolics*, mixtures prepared with seedless grape pomace samples showed a higher value compared with the mixtures with seeds grape pomace, the values depending on the addition level of grape pomace in maize flour. Different trends for the antioxidant activity of mixtures formulated, depending on the mixture type, were observed. The amount of grape pomace which substituted maize flour influenced more or less the antioxidant activity, depending on the phenolic compounds that are present in each sample extract. Some of the obtained results were disseminated by presenting them as a poster at international conferences, so: **(1)**. Mironeasa, S., Ungureanu-Iuga, M., Batariuc, A., Coțovanu, I., Mironeasa, C. (2023). *The impact of two different drying methods on the polyphenolic content and antioxidant activity of grape pomace*, 4th International Conference on Food Bioactives and Health, Prague, Czech Republic, 18-21 September, 2023, **(2)**. Mironeasa, S., Batariuc, A., Coțovanu, I., Codină, G.G., Mironeasa, C. (2023). *Grape pomace characterization for future valorization in extruded snacks*, 3rd Food Chemistry Conference: Shaping a Healthy and Sustainable Food Chain through Knowledge, Dresden, Germany, 10-12 October 2023, **(3)**. Batariuc, A., Mironeasa, S., Ungureanu-Iuga, M., Mironeasa, C. (2023). *Characterization of maize flour-grape pomace mixtures for snack production*, 23rd SGEM GeoConference - “Green Science for Green Life”, Vienna Austria, 28 Nov - 1 Dec, 2023, **(4)**. Mironeasa, S., Batariuc, A., Ungureanu-Iuga, M., (2023). *Assessment of the fiber content of grape pomace-maize flour mixtures used to produce high-fiber snacks*, accepted for presentation at the 9th Edition of the International Conference Biotechnologies, Present and Perspectives, 15th of December 2023, Suceava, Romania. Also, the results regarding the fiber content from studied samples were presented at the workshop Sustainable Food: Trends and Opportunities, “Ștefan cel Mare” University of Suceava, Faculty of Food Engineering, Suceava, Romania, **(5)**. Coțovanu (Verniceanu) I., Mironeasa S. (2023). *Grape pomace as a fiber source in extruded products*.

The mixture's *functional properties*, in terms of water retention capacity (WRC) and swelling capacity (SC), showed different values depending on the amount of grape pomace added in maize flour, grape pomace type, seedless or with seeds, grape variety, white or red, and also by the type of drying used. The results regarding functional properties of grape pomace used in mixtures formulation for the extrusion process were presented at the 4th International

Conference on Food Bioactives and Health, Prague, Czech Republic, 18-21 September, 2023, (6). Mironeasa, C., Batariuc, A., Ungureanu-Iuga, M., Coțovanu, I., Mironeasa, S. (2023). *Functional properties of grape pomace used in the extrusion process*, 4th International Conference on Food Bioactives and Health, Prague, Czech Republic, 18-21 September, 2023. Functional properties of the mixtures prepared highlighted considerable differences between samples due to the milling processes of grape pomace which produce physicochemical changes in the features of the material. Different particle-size fractions of grape pomace have different chemical compositions and properties, and these influence the functionality of the mixtures to which they are added. The high values obtained for WRC and SC may be due to the high soluble dietary fiber from mixtures, since these functional properties are determined by the content in water-soluble-fiber-components of material (Sosulski & Caden, 1982), or due to the other components, such as lignin which has water affinity (Lopez et al., 1996). The structural properties of the fiber have a stronger effect on the water-binding capacity of fiber than its chemical composition (Robertson & Eastwood, 1981). The results obtained suggest that the functional properties of mixtures formulated for extrusion are dependent on various factors and grape pomace properties.

The *molecular characteristics* of mixtures, evaluated by using FTIR spectroscopy technique allow for obtaining spectra that present some characteristic bands of individual components. Valuable information about the chemical composition, including both primary and secondary metabolites, of the investigated samples was provided by these bands. FTIR spectra for *maize flour* revealed the distribution of nutritional components, such as moisture, proteins, lipids, ash, carbohydrates, starchy polysaccharides, and also amide I, amide II, amylose, and amylopectin at typical bands of individual components. Compared to maize flour, the mixtures formulated identified the functional groups of the bioactive compounds, different phenolic compounds, fatty acids, polysaccharides, lignins, pectins, and organic compounds, such as sugars, alcohols, and organic acids due to the presence of these constituents in grape pomace. The results regarding molecular characteristics of grape pomace were presented at Elsevier conference, 3rd Food Chemistry Conference: Shaping a Healthy and Sustainable Food Chain through Knowledge, Dresden, Germany, 10-12 October 2023, (7). Mironeasa, C., Ungureanu-Iuga, M., Oroian, M.-A., Mironeasa, S. (2023). *Use of ATR-FTIR spectroscopy technique for the estimation of grape pomace compounds*. Overall, all of the mixtures show similar spectra with peaks occurring in similar locations. Compared to maize flour, there is a reduction or a rise in peak intensity that is approximately proportional to the grape pomace concentration added in mixtures. Moreover, the molecular characteristics are linked to grape pomace type, seedless or with seeds, and grape variety, white or red, but no differences were observed between the samples with grape pomace dried in an oven and those with grape pomace lyophilized.

The research was continued only for mixtures containing oven-dried grape pomace, as there, overall, were no significant differences between oven-dried and lyophilized samples from the characteristics point of view evaluated. This solution was also decided due to the high costs involved in drying grape pomace by lyophilization. Also, because there was no registered remarkable difference between the snack features obtained with different die diameters, and due to high material consumption, we decided to continue the experiment only with the die with 2 mm in diameter.

According to the work plan, it was *purchased the laboratory extrusion equipment*, put in service and training by the C. W. Brabender Instruments, Inc. representant, and S.C. NITECH S.R.L. representant.

The results regarding *preliminary investigations for exploring and selecting the processing parameters and system parameters on mixtures formulated* revealed the influence of extrusion processing parameters, such as feed moisture, feed rate, screw speed, temperature, and die dimension, and also of system parameters, pressure, torque, and specific mechanical energy on finite products. In this sense, these findings were disseminated at the 23rd SGEM GeoConference – “Green Science for Green Life”, International Scientific Conference on Earth

and Planetary Sciences SGEM, Vienna Austria, 28 Nov - 1 Dec, 2023, (8). Coțovanu, I., Mironeasa, S., Ungureanu-Iuga, M., Mironeasa, C. (2023). *Effect of extrusion parameters on the extruded products' features*, by presenting as a poster. Additionally, the manuscript (9). Coțovanu, I., Mironeasa S., Ungureanu-Iuga, M., Mironeasa, C. (2023). *Effect of extrusion parameters on the extruded products' features*, for Proceedings of International Multidisciplinary Scientific GeoConference: SGEM, 2023, was uploaded, being in the Peer Review process.

By manipulating the *extrusion processing parameters*, feed moisture (12 - 18%), feed rate (15 - 20 rpm), screw speed (130 - 170 rpm), barrel temperatures (25 - 180°C), die dimension (ø2-3 mm), die temperature (160 - 180°C) and extrusion system parameters, pressure, torque, and specific mechanical energy, a wide range of tests was performed to achieve expanded products like snacks with desired characteristics. Some of the resulting products can be seen in Figure 1. Also, the feed mixture characteristics were taken into account. The results of these tests allowed us to establish the processing conditions. Additionally, the changes produced by extrusion cooking were taken into account, findings which were reported in the publication article in red zone (Q1), (10). Mironeasa, S., Coțovanu, I., Mironeasa, C., & Ungureanu-Iuga, M. (2023). *A Review of the Changes Produced by Extrusion Cooking on the Bioactive Compounds from Vegetal Sources. Antioxidants*, 12(7), 1453 (Q1). As well, a synthesis of the results was presented at the workshop: Current Trends in Research and Dissemination of Results in the Field Food, “Ștefan cel Mare” University of Suceava, Faculty of Food Engineering, Suceava, Romania, (11). Mironeasa S. (2023). *Extrusion technology in food processing*.

The *extrusion of formulated samples* with different levels of grape pomace (10-40%) performed *at established processing conditions*, highlighted that expanded products has with various characteristics, depending on temperatures along the barrel section and die temperature, screw speed, feeder speed, pressure, and specific mechanical energy. The collected expanded products were dried up to a final average moisture of 4-6% (w.b.) and stored until analysis.



Figure 1. Extrudates snack products results from different tests

As deliverables in the monitoring an internal evaluation of the project activities, the website of the project (<https://fia.usv.ro/cercetare/inadext>) has been actualized, the scientific, and technical report, and the financial report for the second stage was done. Also, the audit report for this second stage was achieved.

In conclusion, *all the activities have been carried out, and the estimated results and the deliverables have been achieved.*

Abstract

The project entitled *Innovative approach to developing value-added snack products through extrusion technology* focuses on obtaining new snack products by valorization of grape pomace for superior health properties in extrusion technology. Using grape pomace and maize flour in new snack formulas can enhance the nutritional content of extruded snacks. The improvements in the nutritional profile of extruded snack products are highly desirable in the food industry.

All the activities (A2.1, A2.2, and A2.3) associated with O2 objective from the second stage, and also, activity A2.1, according to Annex II/Additional act 1/2022 of Contract no. PCE 60 / 2022, was achieved and the estimated deliverables were obtained.

The amino acids content from grape pomace and maize flour were determined. As well, a feed mixture for extrusion was prepared and their physico-chemical, molecular characteristics, and functional properties were assessed. The mixtures, including 33 samples, were prepared by partially substituting maize flour with 10, 20, 30, and 40% seedless grape pomace (SGP) or grape pomace with seeds (GP), from white (W) or red variety (R) variety, dried in a convective oven (O) or lyophilized (L). A database with the results obtained for moisture, acidity, colour parameters, ash, lipids, protein, total dietary fiber content, starch gelatinization, total phenolics, antioxidant activity, molecular characteristics, functional properties, water retention capacity, and swelling capacity was created. After the acquisition of the single-screw laboratory extruder (KE19/25, Brabender, Duisburg, Germany), preliminary investigations for exploring and selecting the extrusion processing parameters and system parameters on mixtures formulated were performed. A database with variable levels tested in different kinds of extrusion processing was created. Then, the extrusion of formulated samples at established processing conditions, dried collected snacks and stored them until analysis was performed. As well, a database on variable levels established and used in the experimental design to obtain extrudates products was created.

The extrusion equipment was acquired, and put in service, and the training was performed by the C. W. Brabender Instruments, Inc. representant, and S.C. NITECH S.R.L. representant.

The website of the project (<https://fia.usv.ro/cercetare/inadext>) has been actualized.

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