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ASSESSING OF MAIZE-BASED SNACKS FORMULATED WITH WHOLE AND SEEDLESS WHITE GRAPE POMACE

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ABSTRACT

Grape pomace is a winery by-product with a high biological value that can be valorized in snacks production. This work aimed to explore the impact of white grape pomace type, seedless (SGPW) and with seeds (GPW) and addition level (10-40%) on the chemical, antioxidant, color, texture, and sensory acceptability of maize snacks obtained through extrusion by means of Response Surface Methodology. Furthermore, the optimal addition level for each grape pomace type was selected and the optimal samples were characterized from a molecular point of view. The results showed that the protein, lipids, fiber, ash, total polyphenols, and antioxidant activity increased proportionally with the addition level, while the cutting force and L* decreased. The optimal samples were found to contain 29.66% SGPW or 29.22% GPW and exhibited enhanced nutritional profile and antioxidant activity compared to maize snacks used as control. The acceptability of the product was satisfactory (>7 score), which confirmed the opportunity to include these ingredients in extrusion to obtain functional snacks. The molecular characterization of the optimal samples revealed changes in absorbances intensities and the presence of additional compounds compared to the control. These results can be of real interest for producers who want to develop novel products with functional benefits and for consumers aware of a healthy diet.

1. Introduction

Extruded corn-based snacks are popular among consumers, and their consumption has raised exponentially worldwide over recent years (Asif et al., 2023). Even if they gained wide-ranging acceptability in all age groups of consumers, these snacks are often lacking protein and fiber, having a deficiently nutritional content (Ačkar et al., 2018). Also, they present a high glycemic index due to high starch and sugar content. The World Health Organization (WHO) has recommended reducing the sugar and carbohydrate contents in such products with high glycemic index. One way to combat the negative consumers health effects of snacks is the addition of vegetable byproducts, recognized as sources of valuable ingredients.

Extrusion technology is promising in developing customized ready-to-eat foods like snacks products, and it has future potential to incorporate functional ingredients, and can contribute to sustainable food production by utilizing byproducts of food industries (Mandliya et al., 2024). Researchers have explored the incorporation of various vegetable byproducts into extrudates. One of this is grape pomace generated by the wine industry which possesses several health and technological benefits (Iuga and Mironeasa, 2020; Lavelli et al., 2016). The chemical composition of the different anatomical parts of the grape byproducts, of the species *Vitis Vinifera* L. depends on the variety, cultivation system, soil, and climatic conditions (Radulescu et al., 2023; Iuga et al., 2019), and also on the winemaking process, the pomace obtained after mechanical pressing having a different composition than that obtained after fermentation. The chemical composition of whole grape pomace differs from that of seedless grape pomace. Whole grape pomace is considered a source of dietary fiber, consisting from cell walls polysaccharides, such as hemicelluloses and cellulose, and of pectin and lignin; it also contains protein, fat, minerals, and bioactive compounds such as phenols (Beres et al., 2016; Iuga and Mironeasa, 2020). Various phenolic fractions were identified in matrix of the grape pomace fibers, which makes fiber have antioxidant properties (Saura-Calixto, 1998; Zhu et al., 2015). After wine making, approximately 70% of the phenolics remain in pomace, depending on the variety, the most important being tannins, phenolic acids, anthocyanins, and resveratrol (de la Cerda-Carrasco et al., 2015; Sousa et al., 2014). Grape seeds constituents are represented by fiber, oils, proteins, phenolic compounds, minerals, vitamins, sugars, organic acids, etc. The compounds with antioxidant properties are more abundant in grape seeds than in grape peels and the main phenols identified are flavanols, procyanidins, and phenolic acids (Iuga and Mironeasa, 2020; Tang et al., 2018). The nutritional value of maize-based snacks can be improved via the addition of bioactive compounds, fibers, and other beneficial nutrients of the white grape pomace. Studies on the addition of vegetable pomace into extruded products revealed that raised pomace levels beyond a certain level had a negative impact on the expansion quality parameters (Altan et al., 2009; Bibi et al., 2017; Selani et al., 2014). Recent studies indicated that chokeberry pomace added at a 20% level determined an increase in the content of dietary fiber, ash, total phenolic compounds, phenolic acids, flavonoids, flavanols, anthocyanins, and antioxidant activity, and didn't worsen the physical properties, hardness, and expansion ratio (Gumul et al., 2023). The incorporation of 10% tomato pomace in corn-based extrudates caused a significant reduction of fat and carbohydrate contents of the extrudates, whereas protein, ash, and fiber were remarkably enhanced (Jabeen et al., 2022). The radial expansion ratio decreased at 15% inclusion of cherry pomace into corn starch extrudates, but the extrusion process did not reduced the total phenolic content (Wang et al., 2017).

Consequently, with the tendency of healthier gluten-free snacks and sustainable development, the partial substitution of maize flour with grape pomace using an extrusion cooking process represents a proper alternative to formulating new snacks. This work aimed to develop newly expanded snacks by extrusion cooking of mixtures from maize flour and whole and seedless white grape pomace at different ratios and evaluate the products from nutritional and sensorial points of view.

2. Materials and methods

2.1. Materials

2.2.1. Samples

The white grape pomace obtained from a Romanian research institute was conditioned to obtain a moisture content of <10%. For seedless grape pomace (SGPW) the seeds were extracted manually from the whole pomace (GPW). Maize flour was provided by a Romanian producer. SGPW or GPW (10-40%) was mixed with maize flour and the moisture was adjusted to 15% (wet basis).

A laboratory single-screw extruder (Kompakt extruder KE 19/25, Brabender, Duisburg, Germany) was used for extrusion. The barrel has a 19 mm diameter, a length-to-diameter ratio of 25:1, and a 2 mm nozzle diameter. Snacks were obtained at a constant feeding speed of 24 rpm and a screw speed of 150 rpm. The temperatures in the four zones were 50°C, 95°C, 175°C, and 180°C. After cooling (16 h) the final product was packed in polyethylene bags.

2.2. Methods

2.2.1. Chemical profile

The chemical profile of the snacks was determined according to standard protocols as follows: ash content was measured according to SR ISO 2171:2023, protein content following SR EN ISO 20483:2014, and lipid content using SR 91:2007. Total dietary fiber was determined with a Megazyme kit (K-TDFR-200a 04/17) according to the AACC 32-05.01 guidelines.

2.2.2. Total polyphenols content (TPC) and DPPH antiradical activity

The extract was prepared by mixing the sample (1g) with a solution containing 70% acetone, 28% water, and 2% acetic acid (v/v/v) (10 mL), followed by ultrasonication (1h), and the supernatant was retained. Two extractions were performed and the liquid phases obtained were mixed.

The total polyphenol content (TPC) was measured by the Folin-Ciocalteu method (FAO/IAEA, 2000). The extract (0.2 mL) was mixed with distilled water (0.8 mL), Folin–Ciocalteu reagent 1N (0.5 mL), and sodium carbonate 20% (2.5 mL). Samples were kept in the darkness for 40 min and the absorbance was measured at 725 nm using a Shimadzu 3600 UV-Vis-NIR spectrophotometer (Tokyo, Japan), with gallic acid (GAE) used as a standard.

The antiradical activity (DPPH) was measured by mixing 0.5 mL extract with 0.5 mL of 80% methanol and was added to 5 mL of DPPH solution. After keeping the mix in the darkness at 25°C for 30 min, the absorbance was read at 517 nm, with gallic acid as a standard.

2.2.3. Rapid (RDS), slowly (SDS) digestible and resistant (RS) starch

The measurements of rapid, slowly digestible, and resistant starch were done according to the international AOAC 2017.16 method, using a Megazyme kit (K-DSTRS; Megazyme, Bray, Ireland).

2.2.4. Snacks color

The color properties (lightness – L*, red or green hue – a*, yellow or blue hue – b*) were measured using a CR-400 chromameter (Konica Minolta, Tokyo, Japan).

2.2.5. Texture maximum force at cutting

The cutting force (CF) of the snacks was measured using a TVT 6700 texturometer (Perten Instruments, Hägersten, Sweden), with a Warner-Bratzler shear blade probe. The measurement was made at a speed of 1 mm/s until the sample was completely broken.

2.2.6. Sensory acceptability

The acceptability of the snacks was investigated by 65 semi-trained panelists. Explanations about the 9-points scale used in the evaluation and a brief training were made before product tasting. Water was used as a neutralizer before each test.

2.2.7. FT-IR spectra

The molecular profile of the optimal snacks was assessed by Fourier transform infrared (FTIR) spectroscopy using a Thermo Scientific Nicolet iS20 spectrophotometer (Waltham, MA, USA) in attenuated total reflection (ATR) mode. The spectra were collected in the 4000 to 400 cm^{-1} range, with a resolution of 4 cm^{-1} and 32 scans.

2.2.8. Experimental design and statistics

The effects of the addition level (A) at 4 levels (10, 20, 30, 40%) and type of white grape pomace (B) at 2 levels (seedless - SGPR and whole - GPR) on maize snacks characteristics (protein, ash, lipids, fibers content, TPC, DPPH, SDS, RDS, RS, L*, a*, b*, acceptability and cutting force) were investigated by using response surface methodology (RSM) by means of a D-optimal design. The actual and coded factors values of the design are shown in Table 1. Three repetitions for each experiment were included.

The experimental data for each response was fitted to a polynomial cubic or quadratic regression equation. Model adequacy was evaluated by using a sequential *F*-test, coefficients of determination (R^2), adjusted coefficients of determination ($Adj.\text{-}R^2$), and significant probabilities. The significance of the coefficients of the models was evaluated by using ANOVA at a confidence level of 95%.

The experimental design and the optimization were carried out by using Stat-Ease Design-Expert software (trial version). To determine the optimal levels of the factors, multiple response analysis was applied to the fitted predictive models, and the desirability function was utilized. For this purpose, the following conditions were established for the responses: the protein, ash, a* and b* were kept in range, the lipids, fiber, TPC, DPPH, SDS, RS, acceptability, and L* were maximized, and the RDS and cutting force were minimized.

Table 1. Actual and coded values of the factors

Run	Actual		Coded	
	A-type	**B-level (%)**	**A-type**	**B-level**
1	SGPW	40	{ -1 }	1.000
2	SGPW	20	{ -1 }	-0.333
3	SGPW	30	{ -1 }	0.333
4	GPW	10	{ 1 }	-1.000
5	SGPW	20	{ -1 }	-0.333
6	GPW	30	{ 1 }	0.333
7	SGPW	20	{ -1 }	-0.333
8	GPW	30	{ 1 }	0.333
9	GPW	20	{ 1 }	-0.333
10	GPW	10	{ 1 }	-1.000
11	GPW	40	{ 1 }	1.000
12	SGPW	10	{ -1 }	-1.000
13	GPW	30	{ 1 }	0.333
14	SGPW	30	{ -1 }	0.333
15	GPW	40	{ 1 }	1.000
16	GPW	20	{ 1 }	-0.333
17	SGPW	40	{ -1 }	1.000
18	SGPW	30	{ -1 }	0.333
19	SGPW	10	{ -1 }	-1.000
20	GPW	20	{ 1 }	-0.333
21	SGPW	10	{ -1 }	-1.000
22	SGPW	40	{ -1 }	1.000
23	GPW	40	{ 1 }	1.000
24	GPW	10	{ 1 }	-1.000

XL STAT was used for means comparison between the optimal and control samples by using ANOVA with the Tukey test ($p < 0.05$).

3. Results and discussions

3.1. Influence of factors on snacks quality

The influence of factors on the responses studied is presented in Table 1. The models used for data fitting were suitable, with a R^2 value >84%. DPPH antiradical activity, RS, and CF were fitted to the quadratic model, while the other responses (protein, ash, lipids, fibers, TPC, RDS, SDS, acceptability, L*, a*, and b*) were fitted to the cubic model. All the models proposed had a significance level of $p < 0.01$.

Table 2. ANOVA results for the cubic or quadratic model fitted for the extruded snacks properties

Factor	Protein (%)	Ash (%)	Lipids (%)	Fibers (%)	TPC (mg GAE/g)	DPPH (%)	RDS (%)	SDS (%)	RS (%)	Acceptability	L*	a*	b*	CF (g)
Const.	9.35	1.39	1.73	14.38	31.71	94.51	36.06	2.46	3.23	7.04	57.68	6.86	12.12	631.63
A-Type	0.22**	-0.04**	0.51**	0.25**	3.15**	0.05	0.82**	0.85**	0.94**	-0.01	-1.36**	0.42**	0.38**	30.02*
B-Level	1.11**	0.69**	1.07**	8.61**	-0.02	0.63*	6.41**	-0.07	-0.26**	0.62**	-1.63**	0.12	0.14	-166.26**
AB	0.14**	0.05**	0.33**	0.07	2.76**	-3.15**	-2.15**	-0.17**	0.18**	-0.06*	-0.55**	0.27**	0.54**	61.04**
B^2	-0.02	-0.13**	0.12**	-1.09**	-8.09	-4.42**	1.65**	0.06**	-0.23**	-0.14**	-0.18	-0.14*	0.28**	56.60
AB^2	-0.23**	0.05**	-0.17**	-0.73**	-8.01**		-1.61**	0.04*		0.08*	1.40**	-0.05	0.12	
B^3	-0.19**	-0.23**	-0.01**	-0.13	3.98**		-1.98**	-0.26**		-0.52**	-2.06**	0.68**	-0.67**	
***p*-value**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
R^2	0.99	0.99	0.99	0.98	0.98	0.91	0.98	0.99	0.99	0.86	0.98	0.97	0.95	0.84
Adj.-R^2	0.99	0.99	0.99	0.98	0.98	0.87	0.97	0.99	0.99	0.82	0.98	0.96	0.94	0.81

* - significant at $p < 0.05$, ** - significant at $p < 0.01$

Figures 1-5 confirmed that the predicted data generated by the RSM cubic and quadratic models align well with the actual experimental data, demonstrating a satisfactory correlation between the two. Consequently, the developed models are suitable for predicting the optimal composite extruded snacks properties based on the factors considered.

The addition level of white grape pomace resulted in significant increases in protein, ash, lipids, fibers, TPC, and DPPH proportional with the amount used. The experimental data *vs.* the predicted ones for these responses are displayed in Figure 1. The results are in agreement with previous research which highlighted the enrichment in protein, ash, and especially fiber of wheat snacks as the amount of grape pomace was higher (Alshawi, 2024). The nutritional composition of grape pomace depends on the variety and the presence of seeds (Martins et al., 2017). The increase in dietary fiber content is due to the grape pomace composition formed mainly by fibers like cell wall polysaccharides and lignin (Martins et al., 2017). Pérez-Jiménez et al. (2008) stated that the dietary fiber from grape pomace has a reduction effect on the lipid profile and blood pressure much higher compared to other fiber sources like oat, psyllium due to the synergistic effect of fiber with antioxidant compounds.

Factor A (pomace type) and B (level) influenced significantly ($p < 0.05$) the protein, ash, lipids, and fibers, while their interaction has effects only on protein, ash, and lipids content (Table 1). TPC was significantly affected by A and the interactions between factors, while the DPPH antioxidant activity was influenced only by B factor and the interaction with A. Mildner-Szkudlarz et al. (2012) reported a tenfold increase of polyphenols and a considerably higher DPPH scavenging activity in biscuits enriched with 30% grape pomace. The authors affirmed that the presence of phenols like gallic acid and catechin from grape pomace determined the improvement of antioxidant activity since these compounds have a great antioxidant power (Mildner-Szkudlarz et al., 2012).

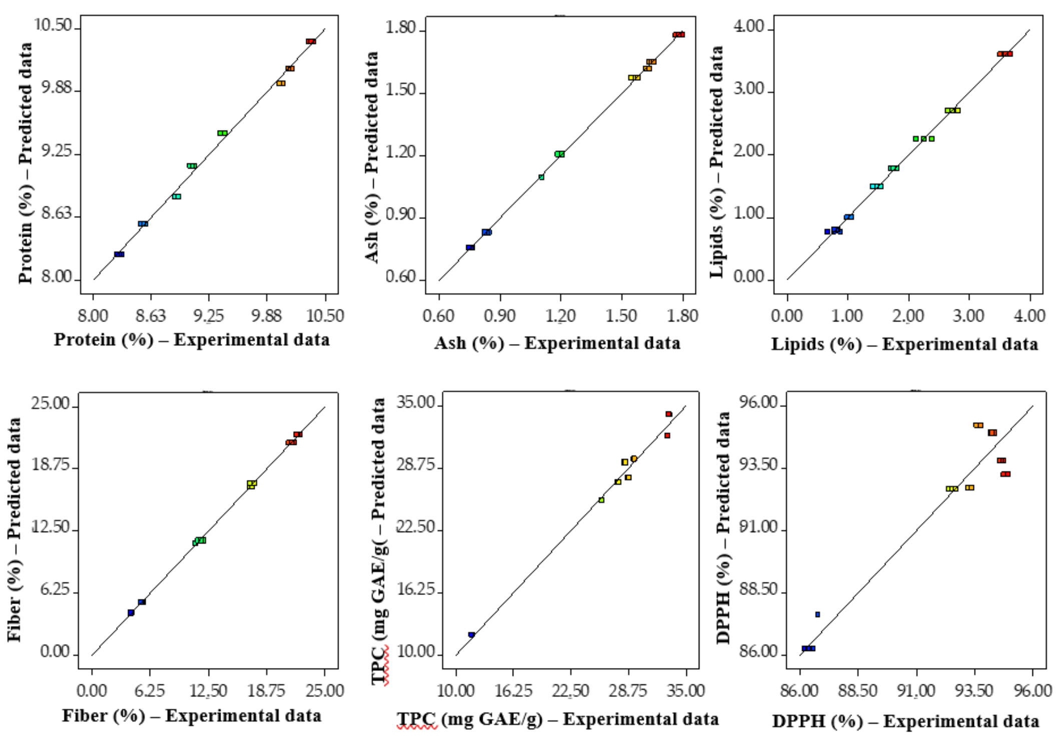

Figure 1. Predicted *vs.* experimental values for the proximate composition, total polyphenols and antioxidant activity

The strong antioxidant activity of gallic acid is given by the inductive effects of its 3 hydroxyl groups (Sánchez-Moreno et al., 1999). Grape pomace polyphenols accomplish the structural criteria for strong antioxidant activity because they contain "either a 3-OH group on an unsaturated C ring, a 2,3-double bond with the 3-OH group and 4-one in the C ring, or an ortho-OH substitution pattern in the B ring, where the OH groups are not glycated" (Rice-Evans et al., 1996). Grape pomace level led to the decrease of SDS and RS content, while the RDS increased. Both factors and their interaction affected ($p < 0.05$) RDS and RS variation, while SDS was not influenced by the addition ($p > 0.05$) level (Table 1). The experimental data *vs.* the predicted ones for starch fractions are displayed in Figure 2. The changes in starch fraction content can be attributed to interactions between polyphenols and starch (Camelo-Méndez et al., 2017). Polyphenols from grape pomace can modify the starch structure and thus its digestibility can be modified (Chi et al., 2017; Sun Miao, 2020).

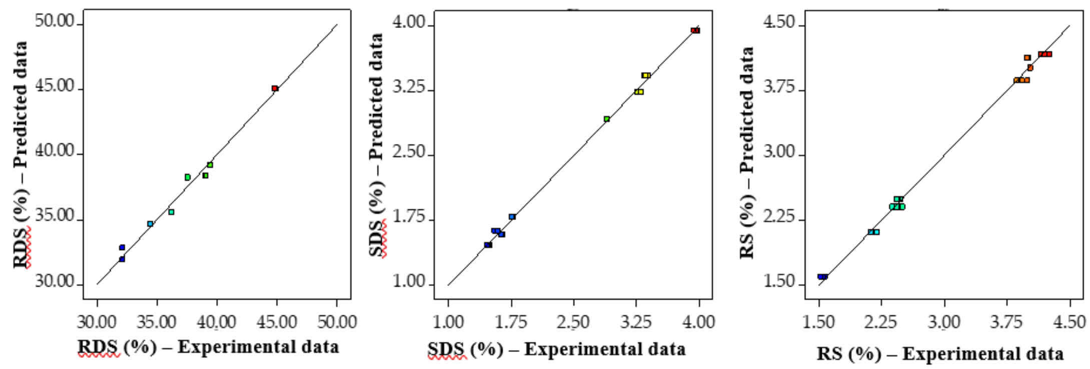

Figure 2. Predicted *vs.* experimental values for the starch fractions

All the color parameters (L*, a*, and b*) were significantly changed ($p < 0.05$) depending on the grape pomace type and the interaction between factors, while the addition level influenced only L* (Table 1). L* and b* values decreased proportional with the addition level increase, while a* increased. Figure 3 represents the dependence of the experimental values *vs.* the predicted ones. Bender et al. (2017) reported intensification of darkness and changes of a* and b* values when white seedless grape pomace was added to muffins. Final product color is influenced by polyphenol oxidase activity, quantity of polyphenols, pH, and ionic linkage strength (Manoj Kumar et al., 2019).

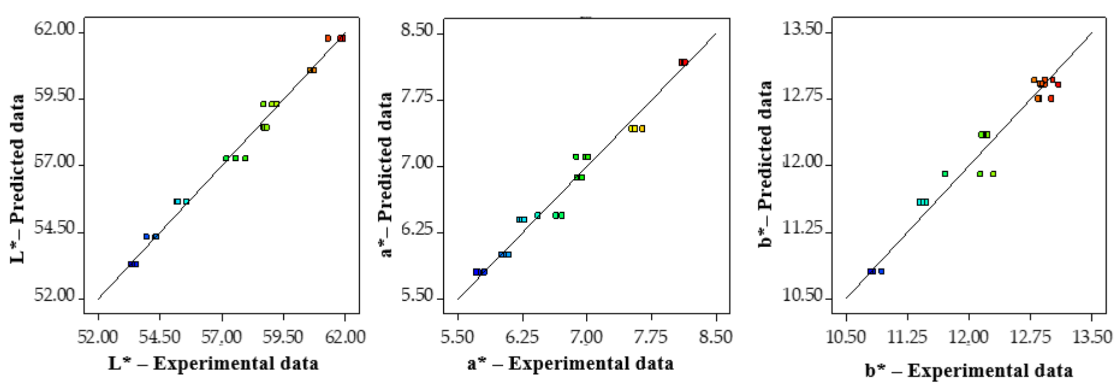

Figure 3. Predicted *vs.* experimental values for the color properties

B factor and the interaction between A and B determined significant variations ($p < 0.05$) of acceptability and CF (Table 1). Grape pomace type (A) has not a significant effect on acceptability ($p > 0.05$). At addition levels up to 30%, the acceptability increased with the raise of the amount incorporated. CF registered a decreasing trend depending on the addition level. A previous study reported a linear reduction of the cut force of rice snacks when cashew apple pomace was added (Preethi et al., 2021). It was stated that the texture of snacks depends on the moisture content, cavity space, sample diameter, compactness of pores, and pore wall strength (Pandiselvam et al., 2019; Preethi et al., 2021). The structural integrity of corn snacks could have been impacted by the interactions between grape pomace and protein which determined changes in cutting force values.

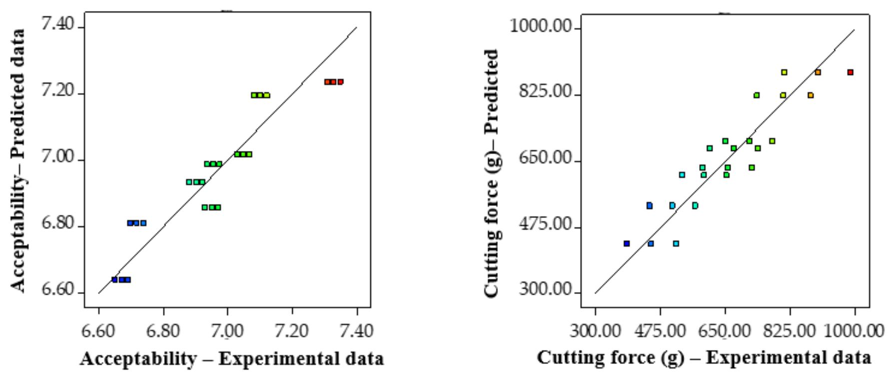

Figure 4. Predicted *vs.* experimental values for the acceptability and cutting force (CF)

Our results regarding product acceptability were in line with those obtained by Kakaei et al. (2019) for corn snacks with pomegranate seeds. They found an increase in overall acceptability probably due to the flavor compounds from grape pomace and/or to the aromatic substances resulting from the Maillard reactions (Kakaei et al., 2019).

The chemical components (protein, ash, lipids, and fibers) were positively correlated among them (Figure 5) and with RDS content. L* and cutting force were negatively correlated with the chemical components listed above.

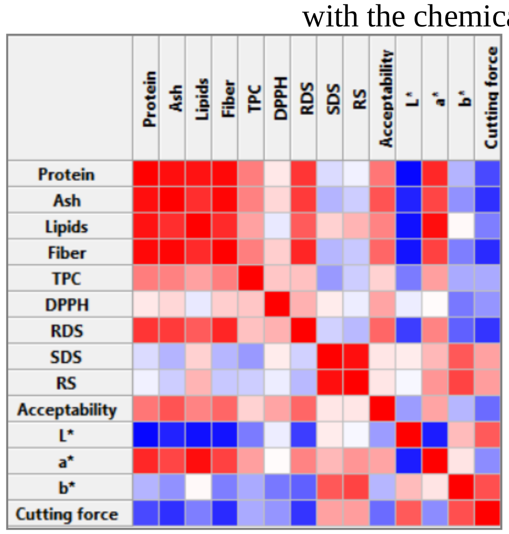

Figure 5. Correlations between the variables (red – positive correlations, blue – negative correlations)

3.2. Optimization

To obtain the optimal addition level for each type of grape pomace, the desirability function was applied. The optimization process revealed that 29.66% SGPW and 29.22% GPW are suitable doses to produce a good quality snack (Table 2). The content of protein, ash, lipids, fiber, SDS, RS, and DPPH was enhanced in the optimal samples compared to the control. The highest TPC level was observed in the O_GPW optimal sample. These results were expected since grape pomace is rich in fibers and polyphenols with antioxidant activity compared to maize flour (Beres et al., 2016; Iuga and Mironeasa, 2020).

Table 2. Characteristics of the optimal samples (predicted values) *vs.* the control

Property	O_SGPW		O_GPW		M	
	Mean	SD	Mean	SD	Mean	SD
Level (%)	29.66	-	29.22	-	0.00	-
Protein (%)	9.44^{b}	0.06	9.91^{a}	0.06	7.61^{c}	0.03
Ash (%)	1.61^{a}	0.01	1.54^{b}	0.01	0.45^{c}	0.01
Lipids (%)	1.48^{b}	0.08	2.64^{a}	0.08	0.35^{c}	0.05
Fiber (%)	16.74^{a}	0.24	16.94^{a}	0.24	0.46^{b}	0.03
TPC (mg GAE/g)	27.80^{b}	0.88	34.44^{a}	0.88	28.85^{b}	0.04
DPPH (%)	95.20^{a}	1.07	93.50^{a}	1.07	38.04^{b}	0.10
RDS (%)	38.15^{a}	0.60	38.05^{a}	0.60	30.03^{b}	0.03
SDS (%)	1.62^{b}	0.05	3.24^{a}	0.05	0.88^{c}	0.03
RS (%)	2.11^{b}	0.07	4.13^{a}	0.07	1.21^{c}	0.01
Acceptability	7.23^{a}	0.08	7.17^{a}	0.08	-	-
L*	58.49^{b}	0.36	55.75^{c}	0.36	78.48^{a}	0.12
a*	6.39^{b}	0.15	7.40^{a}	0.15	0.71^{c}	0.03
b*	11.60^{c}	0.18	12.71^{b}	0.18	21.75^{a}	0.20
CF (g)	536.36^{b}	66.06	636.48^{b}	66.06	1625.43^{a}	147.86

Mean values followed by different letters are significantly different ($p < 0.05$)

The control snack was lighter and had lower a* and higher b* values compared to the optimal samples. These color changes depend on the pigments of the ingredients added and on their chemical composition, especially sugars and amino acids which promote Maillard and other browning reactions. The control sample presented significantly higher cutting force compared to the optimal ones. The presence of fiber from grape pomace likely has a major impact on snacks structure, leading to a lower force needed to break the sample (Różańska-Boczula et al., 2023).

3.3. Characterization of the optimal samples

The characterization of the optimal and control samples from a molecular point of view is presented in Figure 6. Control snacks exhibited the lowest absorbances. The highest absorbances were observed for the O_GPW sample. Compared to the control, the optimal samples exhibited additional peaks at 1106 and 2856 cm^{-1}. The peak at 2856 cm^{-1} is given by the stretching vibration of CH_2 and could indicate the presence of grape pomace cutin, waxes, and cutan (Nogales-bueno et al., 2017). In the study of (Nogales-bueno et al., 2017), the peak at 2924 cm^{-1} was attributed to the C–H stretching vibration of grape pomace structure, while the band at 1741 cm^{-1} was assigned to the stretching vibration of carbonyl (C = O). The protein presence was indicated by the peaks at the protein bands 1647 and 1544 cm^{-1} (Amador-Rodríguez et al., 2019).

The following absorption bands were observed: "Amide II (an N-H bending vibration couples to C-N stretching) (1480–1575 cm^{-1}); N-H bending vibration of primary amines (1580–1650 cm^{-1}); Amide I absorption (predominantly the C=O) (1600–1700 cm^{-1}), and the C=O stretching of triglycerides or alkali ester (pectin) (1745–1740 cm^{-1})" (Amador-Rodríguez et al., 2019), similar with previous reported results. The presence of pectin and cellulose from grape pomace was indicated by the peaks at 1015 cm^{-1} given by the C-O and C-C stretching vibration and 1245 cm^{-1}

given by the C-O stretching vibration (Amador-Rodríguez et al., 2019).

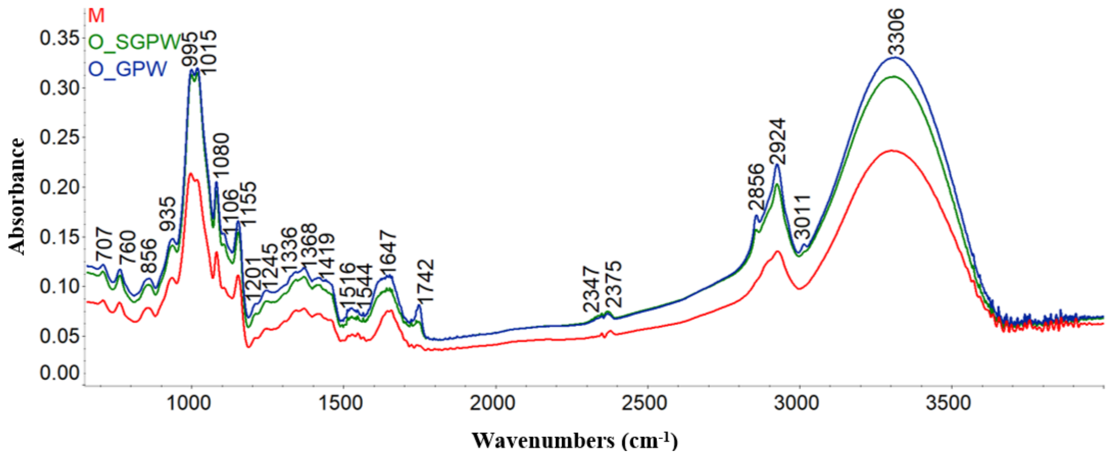

Figure 6. FT-IR spectra of the optimal and control samples

4. Conclusions

Grape pomace valorization in considerable amounts in maize snacks was proved to be feasible since the optimal samples containing 29.66% SGPW or 29.22% GPW presented good acceptability and enhanced nutritional profile. Compared to the maize snacks, the products enriched with grape pomace showed higher nutrients, total polyphenols, and antioxidant activity which may be associated with health benefits. The color and texture of the snacks changed due to the characteristics of the ingredients added, but the acceptability of the product remained in good limits (>7 from 9 points). In conclusion, this paper demonstrated that seedless and whole grape pomace derived from white grape variety processing for wine can be used as an ingredient in snacks production. Further researches regarding the impact of these ingredients on the rheological properties of the mixtures could be assessed.

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