

## Effect of grape skin powder extract addition on functional and physicochemical properties of marshmallow

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### Abstract

#### Keywords:

Grape  
Skin  
Marshmallow  
Antioxidant  
Phenolic

**Introduction.** The present research evaluated the effect of grape skin powder extract addition on functional and physicochemical properties of marshmallow.

**Materials and methods.** To assess the effect of grape skin on marshmallow quality, alcoholic grape skin extracts (GSE) were prepared and introduced in different amounts in marshmallow recipes. The functional properties of zephyrs were estimated by determining the total polyphenol content and antioxidant activity. The microbiological stability of the product was assessed by using agar meat broth. Molds proliferation and morphology of cells from single colonies was studied under microscope.

**Results and discussion.** The effects of grape skin extract (GSE) on marshmallow quality were evaluated. The marshmallow physicochemical properties in terms of moisture and sugar content were affected by GSE incorporation. A directly proportional relationship was observed between the addition of GSE and the moisture content of the marshmallow samples, registering an increase from 15.02 to 15.58% for the samples with 1% and 3% GSE respectively. The sugar content varied in the limits of 14.05–14.21%, being higher for the samples with an increased amount of GSE. Total phenolic content of GSE and marshmallow samples with added GSE was determined as 27.39, 5.11 (1% GSE marshmallow), 6.46 (2% GSE marshmallow) and 7.89 (3% GSE marshmallow) mg/g gallic acid (GAE), respectively. Hydrogen peroxide inhibition capacity and DPPH radical scavenging of marshmallows had increased in proportion to rising GSE level. Antioxidant activity of marshmallows containing 3% GSE was found to be higher (35.72%) than others. The addition of GSE significantly affected the marshmallows color parameters, as the amount of grape skin increased, a more intense purple coloration was observed. The marshmallow containing 2% GSE was most appreciated in terms of sensorial properties. The GSE addition had inhibitory effects on mold population during storage, a higher degree of mold growth reduction ( $p < 0.05$ ) being observed in the sample with 3% GSE after 7 days of storage.

**Conclusions.** The addition of grape skin extract in marshmallow formulation increased the biological value in terms of antioxidant activity and total phenol content, and the consumers' acceptancy.

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## Introduction

It is known that the main wine making by-product is represented by grape pomace, that constitutes about 15/20% of the total grape weight (around 7 million tons) (*Oiv-Statistical-Report-on-World-Viticulture-2018.Pdf*, n.d.). The nutritional quality of grape pomace appears rich in sugars, vitamins, minerals, polyphenols and other macromolecules which are of high interest for the food industry (Bordiga et al., 2019). Due to the rich nutritional compositions and beneficial effects of grape skin on human health demonstrated by numerous researches (Abarghuei et al., 2010; Iora et al., 2015), the interest for the development of new foods based on its use as cookies, various fillings for pastries, drinks, etc. has increased (Acun et al., 2014). Numerous researches have been carried out related to the capitalization of these industrial wastes, including as animal feed, additions in flour mixes for pastry products, ingredients for candy production, sources of biologically active substances with antioxidant potential, etc. (Cappa et al., 2015; Otero-Pareja et al., 2015). The positive effect of grape skin flour on total fibre and ash contents in yogurts and other fermented analogs has been demonstrated (dos Santos et al., 2017). Use of grape skin powder in functional cookies increased their protein and fiber content and showed higher antioxidant potency, higher total phenol and higher retention of hardness during storage (Theagaraian et al., 2019).

The research carried out particularly emphasizes the content of polyphenols in grape skins and their antioxidant capacity. Depending on the grape variety, the total polyphenol content varies within > 20 mg GAE/g of grape skin dry matter for white grapes and >70 mg GAE/g of grape skin dry matter for red grapes (Cvjetko Bubalo et al., 2016; Katalinić et al., 2010), flavonols, catechins and anthocyanins being the main phenolic compounds detected (Antoniolli et al., 2015; Caldas et al., 2018). *Sun and others* have investigated the antioxidant effects of these compounds, and their antitumor and antimetastatic role in preventing breast cancer (Sun et al., 2012), and *Jariyapamornkoon and colab.* showed the positive effect of red grape skin extract on protein glycation (Jariyapamornkoon et al., 2013).

Often to give color and to extend the products shelf life, especially sweet products, manufacturers use dyes and antioxidants of synthetic origin such as carmuazine and tartrazine, etc. which are more intense and stable during products storage (Caleja et al., 2017). However taking into account current trends in the food industry, the need of these researches results from the FAO/WHO strategy of substituting synthetic substances (additives, texture agents, etc.) with natural bioactive components, which is a way to increase food safety and quality, a benchmark in optimized nutrition (McGuire, 2013; Taghvaei et al., 2015) and of diminishing the food waste level (Gustavsson et al., 2011).

Withal, the replacement of synthetic substances with natural ones resulting from the vines processing is a strategic problem, because, unlike synthetic ones, natural substances have fragile molecules, sensitive to food matrix, storage conditions, etc. However, the antiradical and microbiostatic activity of grape skin extracts, rich in polyphenols, is a promising source of alternative solutions for their use in order to replace certain synthetic food preservatives (Katalinić et al., 2010; Shin et al., 2010).

However, as far as is known, the grape variety, food matrix, ingredients and investigated quality parameters in each study are different from those presented in the current study. In the present study the marshmallow prototype was chosen as the food matrix. Marshmallow is a sweet product preferred by all categories of the population, but especially children have always preferred them (Ungure et al., 2013). Due to the fact that many researchers have demonstrated the positive effects of incorporating grape skin into various food products, but less in marshmallows, hence the *purpose* of the present research is to evaluate the effect of grape skin powder extract addition on functional and physicochemical properties of marshmallow.

## **Materials and method**

### **Materials and chemicals**

A standard recipe of marshmallow was used. The ingredients for the marshmallow production were as (Yurchenko et al., 2020): egg whites, agar-agar, sugar, citric acid and grape skin powder.

### **Grape skin extracts**

In order to obtain grape skin extract (GSE), pomace resulting from the production of Merlot wine was used. The grape pomace was initially dried at  $50 \pm 2$  °C until its moisture content reached 5% value. The pomace was then blown to separate the skin from the seeds. The skin of the grapes was minced and hydroalcoholic extracts (50% EtOH, 1:10) were prepared.

### **Marshmallow production**

The marshmallow was prepared using agar-agar, egg whites, sugar, apple puree and water (Yurchenko et al., 2020), with the addition of grape skin hydroalcoholic extract (1.0% and 2.0%, 3.0% of total weight).

The technological process of preparing marshmallows begins with the preparation of apple puree, which involves peeling apples, cutting them into pieces and boiling them with a little water, over a moderate heat until the consistency of the apple pieces is soft. The apple pieces are minced with the a blender and an amount of 30% of sugar is then added. The mixture is put on a moderate heat and mixed until the sugar crystals are completely dissolved.

The apple puree, GSE and the egg whites are placed in the bowl of the mixer and the mixture is foamed starting from the low speed to the higher speed of the mixer. The mixture is foamed until the hard peaks are obtained.

Separately the sugar syrup with agar is prepared: the agar is mixed with water and sugar and put on the fire, stirring constantly until boiling. From the moment of boiling, the syrup is kept on the fire for another 5 minutes until the thin thread syrup is obtained. The syrup then is added to the mixture of egg white and apple puree while the mixer continues to foam the composition. The mixture is beaten until a shiny and firm composition is obtained. Using a pastry bag, the marshmallows are shaped and then allowed to dry for 3-6 hours.

### **Grape skin powder and marshmallow properties**

#### **Moisture content**

The moisture content of grape skin powder and marshmallow samples was determined by oven drying, according to the AOAC, 2005; method 930.15 (Horwitz, 2005).

#### **Sugar content**

The reducing sugars concentration of marshmallows was analyzed by Benedict's, Bertrand's and Fehling standard procedures (Kumar et al., 2014).

### **Titrateable acidity (TA)**

The TA was determined by titration to pH 8.1 with 0.1 M NaOH. Phenolphthalein (0.1%) was used as an indicator (Mutlu et al., 2018).

### **Total Polyphenol content (TPC)**

The total polyphenol content in grape skin powder and marshmallow samples was determined by Folin Ciocalteu method described by *Makkar et al.* (2003) (Makkar, 2003).

### **Antioxidant activity (AA)**

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) solutions were prepared in methanol. The sample solutions (Grape skin and marshmallow hydroalcoholic extracts 100 µL) at varying concentrations (0.1–10 mg/mL) was added to a 0.1 mM methanolic solution of DPPH (3.9 ml) and shaken vigorously. The reaction tubes, in triplicates, were kept in dark, at 30 C for 30 min. Spectrophotometric measurements were done at 517 nm using Hach Lange DR 5000 spectrophotometer. The data are mean±SD (Sharma et al., 2009).

### **Hydrogen peroxide scavenging activity assay**

Hydrogen peroxide inhibition capacity of the GSE and marshmallow samples was determined by replacement titration. Aliquot of 1 ml of sample and 1 ml of hydrogen peroxide solution (0.1 mM) were mixed. Then 2 drops of ammonium molybdate (3%) solution were added, followed by 10 ml of sulfuric acid (2M) and 7 ml of potassium iodide (1.8M). The obtained solution was left to interact for 20 minutes, then titrated with sodium thiosulphate (5.09 mM) until the yellow color disappeared. In parallel, the control sample (without extract) was analysed. The thiosulphate volume expended for titration were record ( $V_1$ –for sample with GSE, and  $V_0$  – for control sample). Percentage of hydrogen peroxide inhibition was calculated as (Nagulendran et al., 2007):

$$\% H_2O_2 \text{ Inhibition} = \frac{(V_0 - V_1)}{V_0 \times 100} \quad (1)$$

### **Color parameters assessment**

In the food science and technology color is traditionally represented using the CIE 1976  $L^*a^*b^*$  or CIELAB color space (Goñi et al., 2017). The influence of the addition of GSE on the chromatic parameters of the marshmallow was evaluated with using the tristimulus Cielab colorimeter. For each sample, individual color parameters  $L^*$ ,  $a^*$  and  $b^*$  were quantified.

Color changes was measured as the modulus of the distance vector between the initial color values and the actual color coordinates. This concept is called total color difference ( $\Delta E$ ). The total color difference indicates the magnitude of the color difference between the control samples and those investigated (Ly et al., 2020).

### **Microbiological analysis**

For each sample, marshmallows were analyzed after 1, 3 and 7 days of storage at  $6 \pm 2$  °C. Appropriate dilutions were made and pour-plated onto selective media. In order to determine the microbiological stability of the product, determinations were performed on agar meat broth for molds proliferation. Each sample was inoculated in triplicate. The selectivity of the growth conditions was confirmed by morphology of cells from single colonies under microscope (Jung et al., 2016).

### Sensory evaluation

In order to perform the organoleptic test, marshmallows were prepared one day in advance. Twelve panelists (aged 24 to 69 years old), participated in this study and appreciated the quality of marshmallows based on the 9-point hedonic scale from "dislike extremely" to "like extremely". For the present research, first was discussed the main quality characteristics of marshmallow. The marshmallow samples were investigated for appearance, texture, taste, flavor, color and overall acceptance (Cano- Lamadrid et al., 2018).

### Statistical analysis

All experiments were carried out in triplicate. The results are given as mean standard deviation (SD). Student's t-test was used for comparison between two means. A difference was considered statistically significant when  $p \leq 0.05$ .

## Results and discussion

### Physico-chemical parameters

Marshmallow samples showed different values of the studied parameters depending on the added GSE concentration.

The following physico-chemical indices were investigated in the experimental samples: moisture content, reducing sugars content and total titratable acidity. The obtained results are illustrated in table 1.

The obtained results (Table 1) indicate that with an increase in the mass fraction of grape skin extract, the moisture content of marshmallows increases as well from 15.05% for the control sample to 15.68% for the sample with 3% GSE addition. This increase in moisture can be explained by the increase, in the same time, of reducing sugars (glucose, fructose) and dietary fibers amount that lead to the increase of marshmallow water binding capacity (Ergun et al., 2010).

**Table 1**  
**Physico-chemical characteristic of the marshmallow**

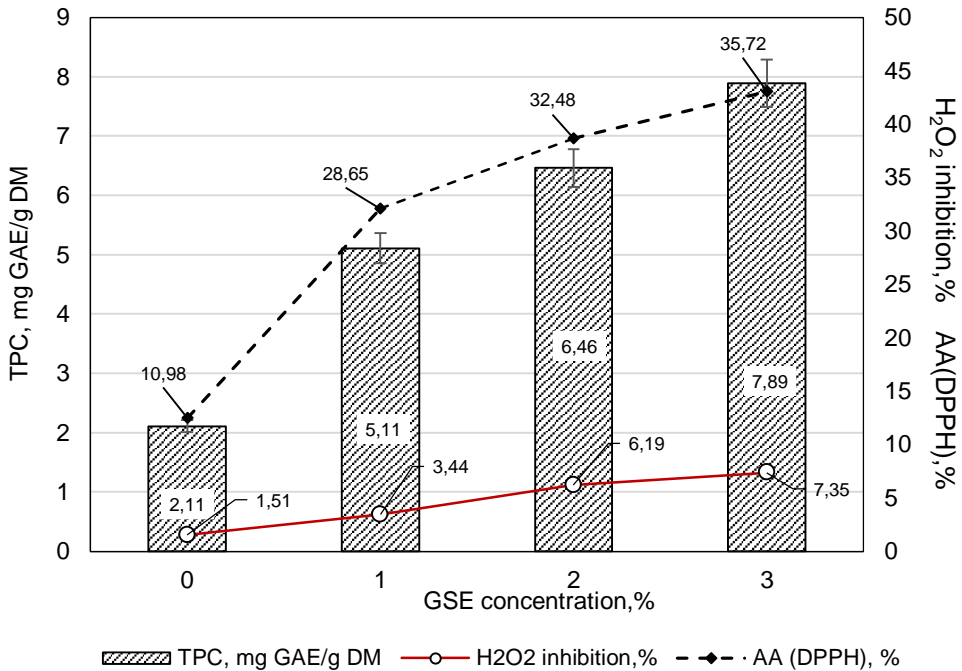
Sample	Moisture,%	Reducing sugar,%	Titratable acidity, mEq/l
Control sample	15.02±0.19	14.05±0.24	7.63±0.11
Marshmallow – 1% GSE	15.21±0.21	14.11±0.16	7.80±0.09
Marshmallow – 2% GSE	15.45±0.15	14.15±0.18	7.82±0.13
Marshmallow – 3% GSE	15.68±0.23	14.21±0.22	8.01±0.08

The fact that the titratable acidity of marshmallow samples increases from 7.63 to 8.01 with increasing GSE concentration is confirmed by the presence of three major acids in grape skin (Le Moigne et al., 2008). The presence of organic acids from natural plant extracts has been associated with the possibility of extending the shelf life of sugar confections like fruit paste, jelly candy and others (Anand et al., 2013).

### Total Polyphenol content and Antioxidant activity

In the context of today's nutrition tendency, when people opt for healthier products with a high biological value (Cucinotta, 2018) it is proposed to determine the total polyphenol content and antioxidant effect of obtained marshmallow with added GSE using the value of the ability to inhibit hydrogen peroxide and the DPPH assay.

The values obtained are indicated in figure 1.



**Figure 1. Influence of the total polyphenol content on the antioxidant activity of marshmallow samples**

The total content of polyphenols in grape skin powder is  $27.39 \pm 0.41$  GAE/g DM, data which are similar to those in the literature, which ranges between  $22.2 \pm 9.95$  mg GAE/g and  $45.0 \pm 26.3$  mg GAE/g depending on grape variety, number of extractions and used solvent (Katalinić et al., 2010; Negro et al., 2003). The incorporation of grape skin in the marshmallow formulation has the effect of increasing the polyphenol content in the investigated samples from  $2.11 \pm 0.29$  mg GAE / g DM for the control sample to  $7.89 \pm 0.23$  mg GAE / g DM for the marshmallow sample with 3% GSE. The same effect of the addition of grape skin on food matrices has been demonstrated in the case of its use in formulations of candies (Cappa et al., 2015; Mutlu et al., 2018), sausages (Ryu et al., 2014), cookies (Acun et al., 2014), etc. In the case of the mentioned products, the positive effect on the total polyphenol content was higher due to the incorporation of GS in the form of powder and not of extract.

In the present study, tests were also performed with the direct use of grape skin powder, but these samples did not have a uniform color distribution, their surface being with purple

dots, and the consistency was also negatively affected, respectively they have were rejected from a sensory point of view.

The antiradical activity of plants is largely due to polyphenols (Pulido et al., 2000). From the data presented in figure 1 it can be seen that the amount of polyphenols increases with increasing GSE concentration in the marshmallow samples. At the same time, the amount of polyphenols is directly proportional to the antioxidant activity of the products.

It is known that grape skin has an enormous antioxidant potential (Dordoni et al., 2019). As presented in figure 1, marshmallow samples with added GSE showed hydrogen peroxide inhibition activity. Many common and life threatening human diseases have free radical reactions as an underlying mechanism of injury. Hydrogen peroxide can cross cell membranes rapidly and form hydroxyl radical and this may be the origin of many toxic effects (Rani et al., 2015). The inhibition of H<sub>2</sub>O<sub>2</sub> by marshmallow samples with added GSE may at least partly result from its antioxidant and free radical scavenging activity. The H<sub>2</sub>O<sub>2</sub> inhibition level of marshmallow with 3% GSE rose to 7.35% comparing to 1.51% for control sample.

DPPH is another free radical which is reduced in the presence of an antioxidant molecule. Based on results presented in figure 1, we can say that as a rule the addition of ethanol grape extract to marshmallow showed higher antioxidant activity values than the control sample. Based on the DPPH assay, the radical scavenging activity of the marshmallow samples with added GSE were 28.65±0.21% for the sample with 1% GSE, 32.48±0.19% and 35.72±0.14% for the sample with 2% and 3% GSE respectively comparing to 10.98% for the control sample. It worths mentioning that the DPPH radical scavenging activity of the GSE was 89.71%. Rockenbach et al., 2011 mentioned values of 16,925 – 3640 µmol Trolox equivalents /100 g for the DPPH assay for different grape varieties (Rockenbach et al., 2011).

### Color parameters

Results concerning the color analysis showed that as a result of the different rates grape skin addition, significant difference was noticed between L\*, a\* and b\* values. Data on the color parameters values (L, a, b) of marshmallow are shown in Table 2.

**Table 2**  
Color parameters values of marshmallow with added grape skin extract

Sample Parameter	Control sample	Marshmallow – 1% GSE	Marshmallow – 2% GSE	Marshmallow – 3% GSE
L*	97.56±1.12	91.00±0.83	81.27±0.41	77.13±0.65
a*	1.21±0.04	4.32±0.11	10.44±0.13	11.52±0.21
b*	-1.34±0.03	-4.27±0.09	-9.31±0.23	-10.37±0.17
ΔE	-	7.83±0.17	20.35±0.09	24.60±0.11
C*	-	6.07±0.06	13.99±0.13	15.50±0.09
H (°)	-	-0.78±0.01	-0.73±0.01	-0.73±0.01

As the amount of grape skin increased, a more intense purple coloration of the marshmallow samples was observed. The control sample was white, with L\* value 97, and tends to decrease by L\*=77 for the 3% GSE sample, this can be explained that at pH higher than 5, brightness slightly decreases, indicating that other colored forms are being formed (Chi et al., 2020). On one hand the red – green parameter (a\*) also shows an increasing

tendency, towards a redder shade. This is due to the anthocyanins present in the grape skin, whose color varies in shades of red – blue (Khoo et al., 2017). The same is demonstrated by the decrease of the  $b^*$  value (blue-yellow), the addition of grape skin giving the products a slightly purple shade. The GSE addition had an influence and on  $\Delta E$  of marshmallow samples, a directly proportional relationship was observed between the increase in GSE addition and the  $\Delta E$  increase. The ( $C^*$ ) – chroma parameter shows the color saturation of the marshmallow samples, i.e. indicates the intensity of the purple color relative to white (Milla'n, 2002). The marshmallows with 3% GSE had a more saturated color. The hue angle (H), is expressed in the scale 00 -3600, considered the qualitative attribute of the color, is the attribute according to which the colors have been traditionally defined as reddish, greenish, etc., and is used to define the difference of a certain color with reference to gray with the same lightness. As for GSE marshmallows' hue angle, the addition of GSE did not significantly influence the parameter values which ranged from -0.78 to -0.73°.

### Microbiological stability

The microbial cells present in the samples to be analyzed on solidified nutrient media formed visible colonies. The total number of mold colonies are shown in figure 2.

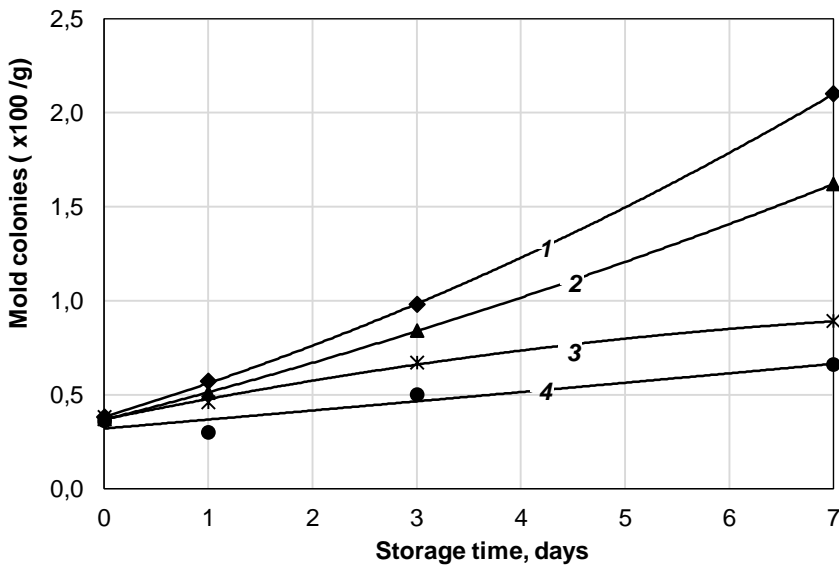


Figure 2. Effect of storage time and grape skin extract addition on microbiological stability of marshmallow.

% GSE:  
1 – 0; 2 – 1; 3 – 2; 4 – 3.

Addition of grape skin extract to marshmallow had inhibitory effects on mould population during storage, a higher degree of mould growth ( $p < 0.05$ ) being observed in the control samples ( $2.1 \cdot 10^2$  colonies/g) as compared to the sample with 3% added grape skin extract ( $0.66 \cdot 10^2$  /g) i.e. 0.5 log reduction in growth in the sample with 3% GPE after 7 days



of storage. The bacteriostatic effect is due to the polyphenols present in the grape skin. The bacteriostatic effect of phenolic compounds has also been demonstrated for *Ocimum basilicum* Leaves Extracts (Ababutain, 2019), cocoa powder (Pina-Pérez et al., 2017, p.), green tea (Zhang et al., 2020), red raspberry (Nikitina et al., 2007), etc. Concerning grape phenols, they showed an antibacterial effect even at 1 and 2.5% concentrations (Furiga et al., 2009; Özkan et al., 2004). Several studies explained the phenols antibacterial activity by the modification of cell membranes permeability (Cushnie et al., 2011), the modification of some intracellular functions induced by hydrogen binding of the phenolic compounds to enzymes (Taguri et al., 2006) or by the modification of the cell wall rigidity with integrity losses due to different interactions between phenols and cell membrane (Negi, 2012).

Length of storage time, showed a concomitant increase in mould population. Seven days storage of control samples increased mould contamination to 0.23 log (Figure 2), indicating an essential effect of storage time on mould growth. The increase of GPE addition to marshmallow formulation showed also a visible effect on mould growth reduction. The main detected mold species were *Aspurgillus versicolor* and *Penicillium islandicum*. The microbiostatic effects of grape skin are also confirmed by previous studies (Hassan et al., 2019).

### Sensory evaluation

Sensory analysis was performed to evaluate the sensory profile of the marshmallow with added grape skin powder extract. The effect of grape skin addition on marshmallow formulation was studied, and 5 quality attributes were evaluated.

Mean scores for liking of color, appearance, flavor (grape notes), taste, texture attributes, and overall liking of samples are presented in Table 3.

**Table 3**  
Mean scores for marshmallow color, appearance, flavor, taste, texture, and overall liking

Sample Parameter	Marshmallow – 1% GSE	Marshmallow – 2% GSE	Marshmallow – 3% GSE
<b>Color</b>	8.2±0.23	9.2±0.24	7.3±0.32
<b>Appearance</b>	7.9±0.31	9.5±0.36	7.1±0.29
<b>Taste</b>	8.1±0.27	8.9±0.45	8.7±0.37
<b>Flavor</b>	7.7±0.26	8.9±0.46	8.1±0.28
<b>Texture</b>	8.4±0.28	8.4±0.27	8.3±0.41
<b>Overall acceptability</b>	8.06±0.27	8.98±0.41	7.9±0.69

Parameters that were most influenced by the addition of grape skin were color and flavor. Due to the anthocyanin content of the grape skin, the marshmallow samples acquired a shade of purple, which in the case of using 3% of GSE was too intense, similar to synthetic dyes, for this reason the panelists gave this parameter 7.3 points. In terms of flavor, color and appearance, the most optimal amount of GSE addition would be 2%, in this case the products had a pleasant color and slightly perceived notes of grape flavor. In general, all the tested marshmallow samples obtained scores above 7.0, on a nine-point hedonic scale, and can be considered as acceptable.

## Conclusion

- The development of marshmallow with grape skin extract is a good strategy to promote the valorization of an industrial waste that has high biological value.
- The formulation of marshmallow with 3% grape skin extract addition led to the best results in terms of total phenol content, antioxidant capacity, color, texture and general acceptability.
- The fortification with GSE increased the total phenol content and antioxidant activity.
- The addition of grape skin extract also achieved other advantages: the reduction of mold growth during the storage and the delivery of beneficial compounds for human health, promoting in the same time the efficient use of a plant material used to be considered an industrial waste.

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