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ORIGINAL ARTICLE

Glutathione-enriched fruit based candies: ıngredient optimization ımpact on storage stability and product quality

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Abstract

In this study, the effects of sodium alginate, gum arabic and whey protein isolate in the production of glutathione enriched fruit-based freeze-dried candies were investigated. Response Surface Methodology was employed to optimize the formulation. Drying efficiency rehydration efficiency, moisture content, color, texture, thermal, and sensory properties were determined to monitor product quality and storage stability. The optimum formulation was determined as 0.99 g/100 g of sodium alginate mixture, 1.05 g/100 g of gum arabic mixture, and 12.26 g/100 g of whey protein isolate mixture according to storage stability, moisture content, and rehydration efficiency. It was observed that WPI positively affected drying efficiency, while sodium alginate had a greater effect on texture compared to other additives. Products containing WPI received higher total acceptability than others. Sodium alginate positively influenced hardness and breakability. Component optimization further increased Tg and had a positive impact on breakability and hardness values than components used alone.

Keywords Freeze drying · Functional candy · Sodium alginate · Gum arabic · Whey protein isolate

Introduction

In recent years, functional fruit-based candies have garnered attention from consumers due to their increased awareness of nutrition and health. Furthermore, there has been a rise in demand for nutritionally appealing candies that meet evolving consumer dietary preferences (Riseh et al. 2022). Freeze-dried fruit-based candies offer the advantage of being prepared without the need for heat treatment, thereby allowing them to be produced safely without compromising the functional properties. However, to achieve the desired texture and other sensory properties in the product, some structure enhancers are required before freeze drying process (Karwacka et al. 2022). The pores formed during the sublimation of the ice in the food matrix and the escape of water create a fragile but spongy matrix, providing the appearance of a product in which the roof matrix of the food is protected without shrinkage. In addition, thanks to the low water activity values obtained by removing moisture, enzymatic and microbiological activity is restricted and safe food production is ensured. The process, which generally causes a significant lightening in the colors of the products, has a protective effect against enzymatic and non-enzymatic darkening reactions caused by other drying methods (Yosefiyan et al. 2024). However, the resulting spongy structure brings with it negative effects such as high moisture absorption, fracturability and collapse. All these structural problems that occur in fruit-based freeze-dried products are tried to be prevented by using amorphous structural elements (Ciurzyńska et al. 2019). For this purpose, the use of sodium alginate, gum arabic and whey protein isolates has been the subject of many studies.

Glutathione (GSH) is an important low molecular weight water-soluble thiol with a tripeptide structure consisting of cysteine, glutamate and glycine with many biological functions such as antioxidant activity, immunological efficacy and ability to regulate intracellular metabolism (Dolbashid et al. 2018). GSH is the most abundant intracellular and the most important non-enzymatic antioxidant in the body. As

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an electron donor, it reacts directly with reactive oxygen species for peroxide reduction (Cruzat and Tirapegui 2009).

GSH production in the body depends on various factors, such as intake in diets and the amount of cysteine. Since the turnover rate of GSH in the human body can reach a maximum of 65% per day, consumers have turned to GSH supplements to maintain or increase GSH levels (Dolbashid et al. 2018). Maintaining optimal tissues for GSH levels is associated with oxidative stress (Sinha et al. 2018). Therefore, it may play a key role in aging and the pathogenesis of many chronic diseases. It has been reported that even partial depletion of GSH in the body impairs immune function and increases sensitivity to xenobiotics and oxidative damage (Sinha et al. 2018), cancer (Townsend et al. 2003), cardiovascular diseases, arthritis and diabetes, aging (Nuttall et al. 1998), Alzheimer's (Saharan and Mandal 2014), chronic liver disease (Hong et al. 2024), cognitive impairment (Rae and Williams 2017) hypertension (Deponte 2013). infertility in both men and women (Koe and Sabanegh 2014), lupus (Shah et al. 2013), mental health disorders (Zalachoras et al. 2020), multiple sclerosis (Carvalho et al. 2014) and neurodegenerative disorders are associated with GSH deficiency (Aoyama and Nakaki 2013). A potentially important approach to protecting against disease by increasing detoxification capacity is increase of GSH, counteracting disorders associated with GSH depletion (Lushchak 2011).

L-glutathione plays a vital role in the detoxification of metabolic products such as lipid peroxides and xenobiotic compounds such as pollutants, heavy metals and drugs (Allen and Bradley 2011). Additionally, various studies have proven that L-glutathione slows carcinogenesis, weakens the immune response, and eliminates toxic xenobiotics (Roy and Paira 2024).

The present study aimed to devolop a new, nutritious functional fruit based candy by incorporating oral glutathione into a fruit blend of persimmon and orange. To enhance the physico-chemical attributes, sensory appeal, and shelf-life stability of the product, the formulation using sodium alginate, gum arabic, and whey protein isolate is optimized through the response surface methodology.

Materials and methods

Materials

Persimmon (*Diospyros kaki*), also known as Japanese persimmon, and Washington orange (*Citrus sinensis*) were used in this study. These fruits were procured from local producers in Balıkesir province, Turkey. The fruits were stored at 4˚C until the obtainment of pulps. In addition, sodium alginate was supplied from Alfasol, China and gum arabic was supplied from Benosen, France, whey protein was supplied from Green Brand, Turkey and Glutathione was procured from Sigma-Aldrich, California.

Ingredient optimisation and candy preparation

The samples according to the recipes (Table 1) were pulped and frozen in silicone molds at−45 °C for 2 h. The contol sample has fruit premix and glutathion. Frozen premixes were freeze-dried using Harvest Right (USA) at a maximum temperature of 52 °C and a pressure of 0.3 mbar for 22 h.

A response surface methodology (RSM) was selected to optimize the range of experiments. CCD was employed in this study to optimize the ingredient formulation. It was used to calculate the regression equation between inputs and outputs, to search for optimum input variable (factor) levels with the "Gradient Search Method" on these regression equations using the observation values obtained from the experiments.The regretion model was calculated using Eq. (1).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \varepsilon \tag{1}$$

In that equiton Y refers to the response variable, X_i and X_j ferefers to factors. Drying efficiency and rehydration capacity were measured as response variables and two regression equations were established regarding these responses. The factors are amounts of the SA, GA and WPI (Table 1).

Central composite design levels and combinations on that levels were given in Table 1. Thus, the impact evaluation and analysis of the used factors and response variables were carried out.

In order to compare the values obtained in the model with the control sample that does not contain protein and hydrocolloid, the control (C) sample trial plan is placed in the table. Data other than the minimumnd maximum values in the trial models obtained with Central Composite Design were not eliminated in order to examine the diversity of the results. Hereby, it was aimed to analyze potential extreme values and evaluate the test results more comprehensively. In addition, in order to examine the effect of each additive on the premixes, the formulations in which sodium alginate, gum arabic and whey protein isolate were added individually to the premixes are listed in Table 1. In this way, the effect of each additive on premixes when used individually has been investigated in more detail and revealed.

Physico-chemical properties

The physico-chemical properties of premixes and freeze dried candies were monitorized by measurements of

Table 1 Central composite factorial design for fruit based glutathion candies formulation

Sample No	Sodium alginate X_1 (g)	Gum arabic x_2	Whey protein X_3 (g)	Glutathion (g)	Persimmon puree (g)	Orange puree (g)
1	1.5	1	5	1	70.75	70.75
2	4.5	1	5	1	69.25	69.25
3	1.5	3	5	1	69.75	69.75
4	4.5	3	5	1	68.25	68.25
5	1.5	1	15	1	65.75	65.75
6	4.5	1	15	1	64.25	64.25
7	1.5	3	15	1	64.75	64.75
8	4.5	3	15	1	63.25	63.25
9	0.48	2	10	1	68.26	68.26
10	5.52	2	10	1	65.74	65.74
11	3	0.32	10	1	67.84	67.84
12	3	3.68	10	1	66.16	66.16
13	3	2	1.59	1	71.205	71.205
14	3	2	18.41	1	62.795	62.795
15	3	2	10	1	67.00	67.00
16	3	2	10	1	67.00	67.00
17	3	2	10	1	67.00	67.00
18	3	2	10	1	67.00	67.00
19	3	2	10	1	67.00	67.00
20	3	2	10	1	67.00	67.00
21	4.5	0	0	1	73.00	73.00
22	0	3	0	1	73.50	73.50
23	0	0	15	1	67.00	67.00
C*	0	0	0	1	74.50	74.50

*C, Control group.

moisture content (MC), water activity (a_w), rehydration capacity (RC) and color.

The moisture content was calculated with the formula in Eq. (2) using the data obtained from the gravimetric change as a result of drying the samples at 105 ± 5 °C until they reached a constant weight.

$$\mathrm{MC} = (\mathrm{W1} - \mathrm{W2}) / (\mathrm{W2} - \mathrm{W3}) \times 100 \tag{2}$$

where W1 is the initial weight of the weighing vessel and the sample, W2 is the final weight of the weighing vessel, and W3 is the weight of the sample and the weighing vessel.

Water activities of freeze dried formulations were measured using a water activity meter (FA-st/1 Model, GBX, France).

The rehidration capacity (RC) data was obtained according to the Jia et al. (2024). After freeze drying weighed samples were put into the tared centrifuge tubes with 50 mL of distilled water. After rehydration process for 24 h, samples were centrifugated using a centrifuge (Elektromag M815A) at 3500 rpm, for 15 min. After the process, the tubes were weighed again and the rehydration capacity was calculated according to the formula in Eq. (3).

$$\mathrm{RC} = \frac{m}{m0} \tag{3}$$

where the m_0: first weighing and m is the final weighing.

The color aspects as L^*, a^*, b^*, Hue (h_0) and chroma (C) data were measured using a colorimeter (Lovibond LC100, Tintometer GmbH, Germany) was calibrated according to the Stewart reflective plate located in the lighting position. Color functions were calculated for illuminant C at 2° standard observer and in the CIELAB uniform color space. Three replicates of each sample were analyzed.

Drying efficiency

Drying efficiency (DE) was calculated according to the formula in Eq. (4).

$$DE\,(\%) = [Final\ weight\,(g) - Initial\ weight\,(g)] * 100 \tag{4}$$

Thermal properties

Thermal properties of glutathion enriched freeze dried fruit cubes, SA, GA, WPI, glutathion were determined using Differential Scanning Calorimeter (DSC 7020, HITACHI High-Tech Corporation, Japan). 10 mg of samples were put into the aluminum hermetically sealed containers. The analysis were achieved at 10 °C /min of heating rate. Heating parameters were for SA, GA and WPI at −60 °C – 250 °C, for glutathion 25–300 °C. For the optimum sample obtained

Table 2 Optimization table

Codes	Factors	Levels (g/100 g)
SA*	X_1	0.99
GA*	X_2	1.05
WPI*	X_3	12.26

*SA, Sodyum alginate, *GA, Gum arabic, *WPI, Whey protein isolat.

with RSM results, temperature values between 30 °C and 250 °C, 5 °C /min heating rate and nitrogen gas were used.

Texture and morphological aspects

Texture properties were determined by measuring hardness and fracturability parameters using a texture analyzer (TA-XT Plus, Stable Micro System, UK). 10 × 10 mm of freeze-dried glutathione cubes were compressed using a three point bend rig probe with a testing speed of 3 mms^{-1}. Hardness value was the initial force (expressed in Newton) required to provide a certain deformation in the candy structure. Fracturability value was defined as the expression of the deformation value in mm caused by the force required to fracture of the sample (Bourne 2002).

Texture characteristics were only determined for samples with a general acceptability score of 4 in order to compare analytical data and sensory data.

Microstructural properties of freeze-dried glutathione candies were determined with micrograph images enlarged at 100 × and 1000 × ratios. Micrograph images were obtained using scanning electron microscope (SEM) (FEI Quanta 250 FEG, USA) 25 (Sakooei-Vayghan et al. 2020).

Sensorial analysis

The panel, which was conducted with 10 trained panelists (3 women and 7 men) was carried out in 3 repetitions. The control group and other samples are coded with random numbers. The prepared sensory analysis form includes 2 features defining appearance features, 4 features defining taste features, 6 features defining texture features and general acceptability. The hedonic scale was used for describing the sensory properties of samples ranges from 1 to 5 points (1 point: Non-existent, 5 points: Too much).

Statistical analysis

The data obtained within the scope of this study was analyzed statistically using the SPSS (V.26) program. The significance of statistical differences was lettered using the Minitab (V.20) program ANOVA-Tukey test.

Table 3 Glass transition points of the samples for begining (Tg_0) and 6 months storage (Tg_6)

Sample No	Tg_0	Tg_6
Control	69.3	56.3
21	70.7	
22	71.8	
23	68.8	
Optimum	74.3	53.5

Results and discussion

Optimisation

An optimization model targeting maximum DE and minimum RC was created using the Minitab Response Optimizer (Microsoft) module (Table 2).Comparing different prepared formulations the suggested statistical quadratic model is as follows:

The effect of independent variables on DE and RC are presented Eqs. (5) and (6).

$$\begin{aligned} Drying\,efficiency\,(Y^1) = {} & 13,54 + 1,29\,X_1 - 3,50\,X_2 \\ & - 0,982\,X_3 + 0,055\,X_1X_2 \\ & + 0,804\;X_2X_2 + 0,0265\,X_3X_2 \\ & - 0,123\,X_1X_2 - 0,0267\,X_1X_3 \\ & + 0,062\,X_2X_3 \end{aligned} \tag{5}$$

$$\begin{aligned} Rehydration\,capacity\,(Y^2) = {} & 10,55 + 0,19\,X_1 - 3,50\,X_2 \\ & - 0,982\,X_3 + 0,055\,X_1^2 \\ & + 0,804\;X_2^2 + 0,0265\,X_3^2 \\ & - 0,060\,X_1X_2 + 0,0080\,X_1X_3 \\ & + 0,0990\,X_2X_3 \end{aligned} \tag{6}$$

where Y is the response, X represents the factors studied with linear terms (Xn), and their two-way interaction terms (XnXm), and quadratic terms Xn^2 (Table 3).

Physico-chemical aspects

The moisture content of the premixes was determined between 63.5 and 78.9%. ($p < 0.05$) (SM). The control group has the highest moisture value. It was determined that as the alginate concentration increased, the moisture of the products decreased ($p < 0.05$) and gum arabic had a minimal impact on moisture levels ($p > 0.05$). Conversely, increasing the amount of WPI had a more detrimental effect on moisture content compared to GA ($p < 0.05$). After the drying process, moisture contents decreased and ranged from 1 to 2.2% in the products (Table 4).

Silva-Espinoza et al. (2021) reported the moisture content of freeze-dried orange candies as 3.63–5.30%. Also Ciurzyńska et al. (2022) reported that the moisture content of fruit snacks, which was between 70.32 and 81.07%

Table 4 Physico-chemical, color and texture data of freeze dried glutathione candies

Candies No	MC (g/100 g)	L*	a*	b*	C	h°	DE (%)	RC (%)	a$_{W}$	Fracturability (mm)	Hardness (N)
1	1.9±0.1^{a}	80.3±3.5^{a}	11.2±2.1^{b}	41.8±0.2^{a}	43.3±1.1	75.1±2.1	21.45±0.05	4.84±0.01	0.18±0.11	7.77±2.1	35.8±2.5
2	1.7±0.2^{a}	83.2±3.2^{a}	8.5±1.9^{bc}	40.0±0.5^{a}	40.9±0.8	78±2.5	23.18±0.03	6.12±0.05	0.19±0.02	9.23±0.8	58.28±3.1
3	1.3±0.4^{a}	79.0±4.1^{a}	11.3±2.0^{b}	38.4±0.2^{b}	40±1.2	73.5±3.1	21.81±0.02	4.6±0.01	0.2±0.00		
4	1.6±0.4^{a}	76.6±3.1^{a}	10.8±2.2^{b}	36.6±0.1^{c}	38.2±0.9	73.5±2.9	22.91±0.11	4.88±0.02	0.17±0.01		
5	1.3±0.4^{a}	82.7±4.1^{a}	7.1±3.0^{bc}	35.6±0.2^{c}	36.3±1.0	78.7±0.9	25.93±0.05	1.68±0.03	0.15±0.01		
6	1.0±0.5^{a}	80.8±3.1^{a}	8.3±2.4^{bc}	38.4±0.3^{b}	39.3±0.7	77.8±0.5	26.97±0.15	2.56±0.02	0.15±0.02	9.47±0.5	63.7±1.8
7	1.4±0.3^{a}	79.5±4.3^{a}	9.5±2.1^{bc}	34.1±0.1^{d}	35.4±0.6	74.4±1.2	27.63±0.12	2.78±0.02	0.16±0.01		
8	1.3±0.4^{a}	79.0±3.3^{a}	8±2.1^{c}	38.5±0.2^{b}	39.4±1.2	78.3±0.9	27.62±0.05	1.29±0.02	0.14±0.01	8.75±1.8	52.48±2.1
9	1.1±0.5^{a}	80.3±3.1^{a}	10.2±1.9^{b}	39.1±0.2^{b}	40.4±1.0	75.4±1.5	23.63±0.01	3.94±0.04	0.17±0.01		
10	1.4±0.4^{a}	78.9±2.1^{a}	9.7±1.6^{bc}	34.1±0.1^{d}	35.5±1.2	74.2±1.3	26.72±0.02	4.98±0.03	0.14±0.01		
11	1.0±0.5^{a}	79.1±3.1^{a}	9.3±1.5^{bc}	37.1±0.2^{bc}	38.2±1.5	75.9±1.6	24.46±0.01	3.21±0.02	0.17±0.02		
12	1.4±0.4^{a}	75.7±3.2^{b}	11±2.1^{b}	33.3±0.2^{e}	35.2±0.8	71.8±1.8	20.5±0.03	6.91±0.03	0.22±0.02	8.91±1.5	87.87±2.3
13	1.4±0.3^{a}	75.3±3.1^{b}	12.2±2.0^{b}	34.8±0.2^{cd}	36.9±0.9	70.9±3.2	19.56±0.1	6.5±0.03	0.21±0.01	9.28±0.8	79.86±1.7
14	1.4±0.3^{a}	83.2±2.1^{a}	6.8±3.1^{c}	31.4±0.^{f}	32.1±0.7	77.8±1.0	29.7±0.01	2.82±0.02	0.11±0.01	9.73±0.5	52.06±1.2
15	1.8±0.3^{a}	79.5±4.3^{a}	9.4±2.1^{bc}	33±0.2^{e}	34.3±1.3	74±2.4	26.07±0.02	2.43±0.02	0.15±0.03		
16	1.5±0.3^{a}	79.0±4.2^{a}	9.1±2.7^{bc}	33.5±0.3^{e}	34.2±1.2	73.6±1.6	26±0.11	2.14±0.01	0.14±0.01		
17	1.7±0.3^{a}	78.8±5.1^{b}	9.6±3.1^{bc}	33.2±0.3^{e}	34±2.1	73.9±2.1	25.81±0.08	2.55±0.01	0.15±0.02		
18	1.8±0.2^{a}	79.6±0.1^{a}	8.8±2.0^{bc}	32.8±0.2^{ef}	34.5±1.4	74.2±2.4	26.16±0.02	2.36±0.01	0.16±0.02		
19	1.7±0.3^{a}	79.4±0.1^{a}	9.7±1.2^{bc}	33±0.3^{ef}	34.6±1.2	74±2.3	26.35±0.08	2.07±0.02	0.15±0.02		
20	1.6±0.2^{a}	78.9±0.3^{a}	9.3±0.9^{bc}	32.6±0.3^{ef}	34.2±1.4	74.3±2.5	25.98±0.03	2.19±0.01	0.14±0.01		
21	1.9±0.1^{b}	74.3±0.2^{b}	12±1.2^{b}	34.4±0.1^{d}	36.4±1.2	70.8±1.9	19.39±0.03	4.24±0.01	0.25±0.02	9.42±0.5^{a}	115.46±3.5^{g}
22	2.2±0.1^{c}	69.9±0.4^{c}	16.9±0.8^{a}	36.4±0.1^{c}	40.1±0.7	65±3.1	18.91±1.05	8.16±0.52	0.2±0.01	8.61±1.6^{a}	46.84±1.2^{e}
23	1.9±0.2^{b}	82.0±0.7^{a}	7.8±1.0^{c}	28.0±0.2^{g}	29.1±0.8	74.5±1.9	24.27±0.05	2.55±0.01	0.15±0.01		
C*	1.8±0.1^{b}	74.5±0.4^{b}	13.5±1.1^{b}	35.7±0.5^{c}	38.2±0.8	69.3±2.2	16.96±0.07	3.56±0.02	0.16±0.01	9.01±1.2^{a}	42.21±0.5^{h}
Optimum	1.9±0.1^{b}	79.8±0.6^{a}	8.1±1.2^{bc}	40.4±0.2^{a}	41.2±1.2	78.7±0.9	28.43±0.12	2.18±0.01	0.079±0.01	10.36±0.6^{b}	124.89±0.5$^{\imath}$

*C, Control group, lightness (L*), redness (a*), yellowness (b*) values and hue angle (h^{0}) . There is no statistically significant difference between the same letters in the same column ($P > 0.05$). A statistically significant difference was found between different letters in the same column ($P < 0.05$). Hardness and brittleness tests were applied to the samples with a sensory analysis general appreciation score above 4.

before freeze-drying, changed between 0.54 and 2.89% after freeze-drying. The moisture content of vegetable snacks produced using sodium alginate and dried apple pulp powder is between 89.3 and 93.71%. It has been reported that varies between 1.65 and 2.81% after freeze drying (Karwacka ve ark., 2022). Freeze drying increased the shelf life and storage stability of the products by providing a significant reduction in the moisture content of the products.

Water activities of the products varied between 0.079 and 0.25 (Table 4). The lowest water activity was measured in sample containing optimum ingredients. Coded 21 snack contained 4.5 g of sodium alginate has the highest water activity. The water activity decreased by increasing WPI amounts in the samples ($p < 0.05$). The lowest water activity was seen in the product contains higher WPI than others. The water activity value must be maximum 0.6 for microbiological safety. Similar to our results, the water activity values of freeze-dried snacks obtained by adding gum arabic (GA) and whey protein isolate (WP) to tangerine puree was determined from 0.224 to 0.307 (Salvador et al. 2022).

After 6 months of storage, the moisture contents of the control and optimum samples were determined as 2.5% and 4.5%, respectively, and the water activity values were determined as 0.254 and 0.278, respectively.The addition of stabilizer and whey protein caused a slight increase in moisture content (Fig. 1).

Drying efficiency

The drying efficiency (DE) of products has varied from 16.96 to 29.70%. The control sample has the lowest DE value. The possitive corelation has been obtained between WPI amounts and DE ($P < 0.05$) (SM). The highest drying efficiency observed in candies coded 14, which has the highest WPI concentration. While there was a positive relationship between SA/GA and drying efficiency, these increases were not statistically significant and were not as effective as WPI in enhancing drying efficiency.

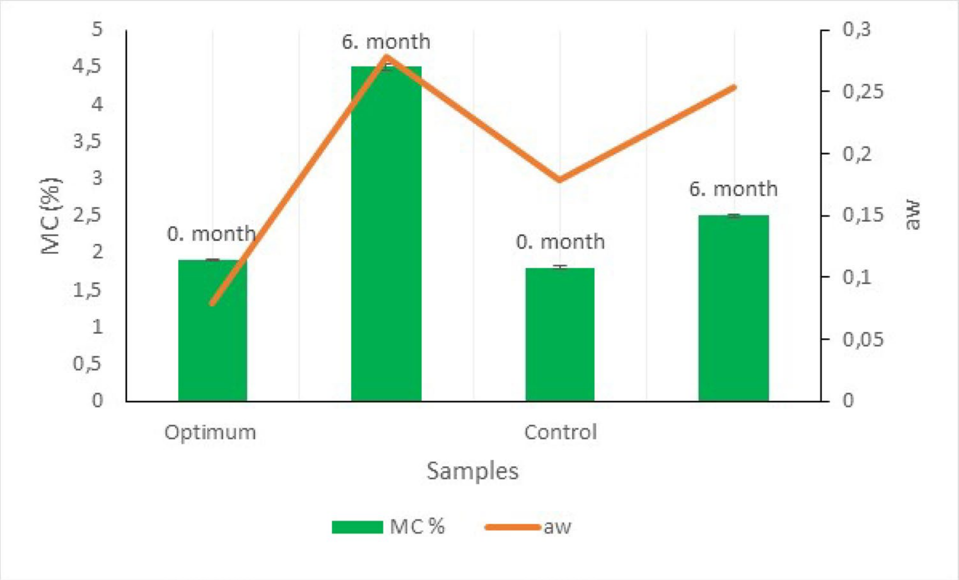


Fig. 1 The alteration of aw and MC (%) values of optimum and control samples after 6 months storage (*n* = 2, experiments were carried out in 3 parallels)

Rehydration capacity

Freeze drying creates a highly porous structure in fruit-based products, preserving their main matrix. While this enhances drying efficiency, it adversely impacts storage stability. Additives in freeze-dried product recipes are often included to stabilize rehydration and increase storage stability, aiming to mitigate the negative effects of high porosity (Fongin et al. 2017).

The sample 8, which included both WPI and SA, exhibited the lowest rehydration capacity while sample 22, containing only GA, showed the highest rehydration capacity. Increasing the amount of WPI did not significantly affect rehydration capacity ($p > 0.05$), while increasing SA decreased rehydration capacity significantly ($p < 0.05$). This suggests potential for improved storage performance compared to other samples.

Increasing the amount of WPI did not significantly affect RC ($P > 0.05$), whereas increasing SA decreased RC significantly ($p < 0.05$), suggesting potential for superior storage performance compared to other examples. Furthermore, as the amount of GA in the snacks increased, their RC also increased ($p > 0.05$). However, the use of GA negatively impacted RC and thus storage stability in the production of fruit-based glutathione-enriched freeze-dried snacks.

Malik et al. (2020) have stated that high gum arabic concentrations increase reconstitution properties by increasing porosity. While the process of restructuring of powder particles with water is a process that is positively affected by gum arabic, it is reported to be a situation that negatively affects the storage stability of some dried products (Mina et al. 2022). It has also been reported that the use of gum arabic at low concentrations does not affect rehydration ability (Mina et al. 2022).

Color aspects

L* values of freeze dried glutathion enriched snacks varied from 69.9 to 83.2 (Table 4). The highest L* value was achieved in the premix with the optimal composition. When SA and GA were used individually in premixes, a decrease in the L* value was observed. Conversely, the use of WPI alone resulted in an increase in the L* value. Moreover, it was found that the incorporation of WPI in freeze-dried samples had a greater impact on the L* value compared to SA and GA ($p < 0.05$).

The a* values of the premixes ranged from 12.6 to 17.8 (Table 4). The a* value decreased in samples containing SA and GA individually, whereas an increase in a* value was observed when WPI was used alone (SM).WPI had a greater impact on the a* value in freeze-dried samples than SA and GA ($p < 0.05$) (Silva-Espinoza et al. 2021). They have reported also that using GA in the production of freeze-dried orange snacks resulted in a decrease in the a* value.

The b* values of the premixes ranged from 26.5 to 36.1 (Table 4). The lowest b* value was recorded in premix sample 3, while the highest b* value was found in the premix with the maximum amount of GA. When SA was used individually in premixes, there was a decrease in the b* value, whereas GA led to an increase in the b* value when used individually. There was no significant change in the b* value when WPI was used alone. However, the inclusion of GA in premixes had a greater impact on b* values compared to WPI and SA ($p > 0.05$). The b* values of freeze-xdried Glutathione candies ranged from 28.0 to 41.8. After freeze drying, the use of GA individually in the mixtures increased the b* value, while the use of SA and WPI individually decreased it ($p < 0.05$). Yellowness was the dominant color of the fruit puree used as research material increased observably due to moisture release during drying. This was determined by the increase in the b* value of all samples after drying ($p < 0.05$).

These results indicated that the freeze-drying process has a significant effect on the color properties of the products and that the addition of different ingredients could changed that effect in the final products.

Thermal properties

The thermal properties of optimized samples were examined using differential scanning calorimetry (DSC). Glass transition points (Tg) are summarized in Table 3. The addition of WPI resulted in lower Tg values than the Tg of the base product. While protein in amorphous structures causes viscosity change in the polymer structure due to the interaction between ion–dipole bonds, it is reported that the transition to glassy state is affected by many factors. Slow cooling, increased time for intermolecular rearrangement of polymer chains, or structural relaxation may cause a decrease in Tg (Khalloufi and Ratti, 2003).

It was determined that the individual use of WPI had a negative effect on the storage stability of the fruit mixture used in the research, therefore its use in optimum concentrations with other additives such as SA and GA could provide a better product stability. Undoubtedly, characteristics of the fruits used, such as sugar/acid ratio and fiber structure, affect the thermal properties of the additives. However, this study showed that it is possible to improve properties known to directly affect storage stability, such as thermal properties by determining optimum mixing ratios. Thermal data of 6 months storage were characterized by a decrease in the glass transition point. It was observed that the structures containing sugar and protein maintained their stability (Table 3.).

Texture profile

Table 4 indicated that hardness values of the freeze dried glutathion encriched snacks. The hardnesses of the snacks were 35.80–124.89N. The highest hardness value was in snack that has optimized composition. A positive and moderate correlation was detected between the amount of SA and hardness values ($p > 0.05$). The increase in hardness values with the amount of GA was not as significant as sodium alginate amount ($p > 0.05$). As the amount of WPI increased, the hardness decreased ($p > 0.05$).

The fracturabilities of glutathione-enriched snacks prepared with different formulations were 7.77–10.36 mm (Table 4). In compliance with the hardness values; the highest fracturability belonged to the sample containing the optimum concentration of components, while the lowest fracturability value was in sample 1. The incorporation of WPI resulted in a decrease in hardness and a slight increase in fracturability values. The use of GA affected hardness and fracturability similarly with a positive correlation ($p > 0.05$). The use of SA increased the hardness of the snacks than fracturability. However, no statistically significant difference was found in terms of their effects on brittleness and hardness values at the dosages of all three additives used ($p > 0.05$). It is thought that these results will set light on concentration and configuration experiments that will increase the hardness value and reduce brittleness for future studies.

The fracturabilities of glutathione-enriched snacks, as detailed in Table 4, ranged from 7.77 to 10.36 mm. Corresponding to hardness values, the sample with the optimal component concentration exhibited the highest fracturability, whereas sample 1 showed the lowest. Incorporation of WPI decreased hardness and slightly increased fracturability values. GA influenced hardness and fracturability similarly with a positive correlation ($p > 0.05$), while SA increased snack hardness more than fracturability. Nevertheless, no statistically significant differences were observed in brittleness and hardness values across the dosages of all three additives used ($p > 0.05$). These findings are expected to guide future concentration and configuration experiments aimed at enhancing hardness and reducing brittleness in snack development.

Ciurzyńska ve ark. (2022), reported that the shear resistance of the products increased with the use of sodium alginate in a study conducted on freeze-dried apple droppers containing sodium alginate. In a study on freeze-dried snacks obtained from frozen vegetable by-products and apple pulp, it was reported that the addition of sodium alginate after freeze-drying increased the hardness values (Karwacka et al. 2022).

Sensorial acceptability and microstructural aspects

The freeze drying process, which leads to high porosity and low tendency to collapse, causes high porosity in the freezing and drying steps compared to other fruit concentration methods. This causes textural properties such as gumminess and stickiness to be less than other drying processes. However, the brix and acid values of fruits are important in characterizing this situation.Conversely, performing processes at temperatures below the glass transition point prevents changes in glass transition and viscosity. This approach helps achieve the desired sensory properties and ensures better storage stability.

The data obtained as a result of sensory analysis were divided into three main groups: taste, texture and appearance (Fig. 2). Snack number 22 had the most intense fruit flavor of all the samples, while snack number 23 had the mildest. Snack number 4 was distinguished by its strong milk flavor, whereas the control sample had the least milk flavor. Snack number 21 was noted for its pronounced metallic taste, conversely control sample has the mildest metallic flavor. Smoothness ratings were highest for snacks 4 and 10 and lowest for snacks 2 and 9. The control sample received the lowest smoothness score, while snacks 1, 5, 6, and 14 were rated the highest for smoothness.

SA contributed to a more uniform structure in glutathione-enriched fruit snacks. However, this contribution did not always align with the perception of smoothness. The snack containing 1.5 g of SA (Code 1) received the highest fibrousness score, whereas the snack with 4.5 g of SA scored the lowest in fibrousness and sandiness. This indicates that lower amounts of SA can create a desirable fibrous texture, while higher amounts effectively reduce sandiness. The addition of WPI enhanced chewiness and stickiness properties. The snack containing 15 g of WPI (Code 22) achieved high intraoral spread scores, demonstrating this effect. The snack with 5 g of WPI (Code 1) was rated the highest for fibrousness, indicating WPI's capacity to improve fibrous texture. The control sample received the highest scores for general acceptability and liking across all sensory attributes. Coded 9 sample received the lowest scores for general acceptability and liking. SA contributed to a uniform structure but higher amounts may result in undesirable properties. WPI significantly improved chewiness, stickiness, and fibrous texture. While the control sample excelled in overall acceptance, certain samples showed limited success in specific sensory parameters.

Scanning electron microscopy was used to determine the macro and micro structure of the snacks with the sensory analysis general appreciation score above 4. Micrographs are given in Fig. 3. The increase in the amount of SA in formulations 1 and 2, which included all the additives used together, led to an increase in fracturability and hardness. (Table 4). SEM image (300 µm) of sample coded 1 containing 1.5% SA, 1% GA and 5% WPI showed homogeneous porosity (Fig. 3A). While other additive amounts remain the same, porosity decreased with a 2% increase in the amount

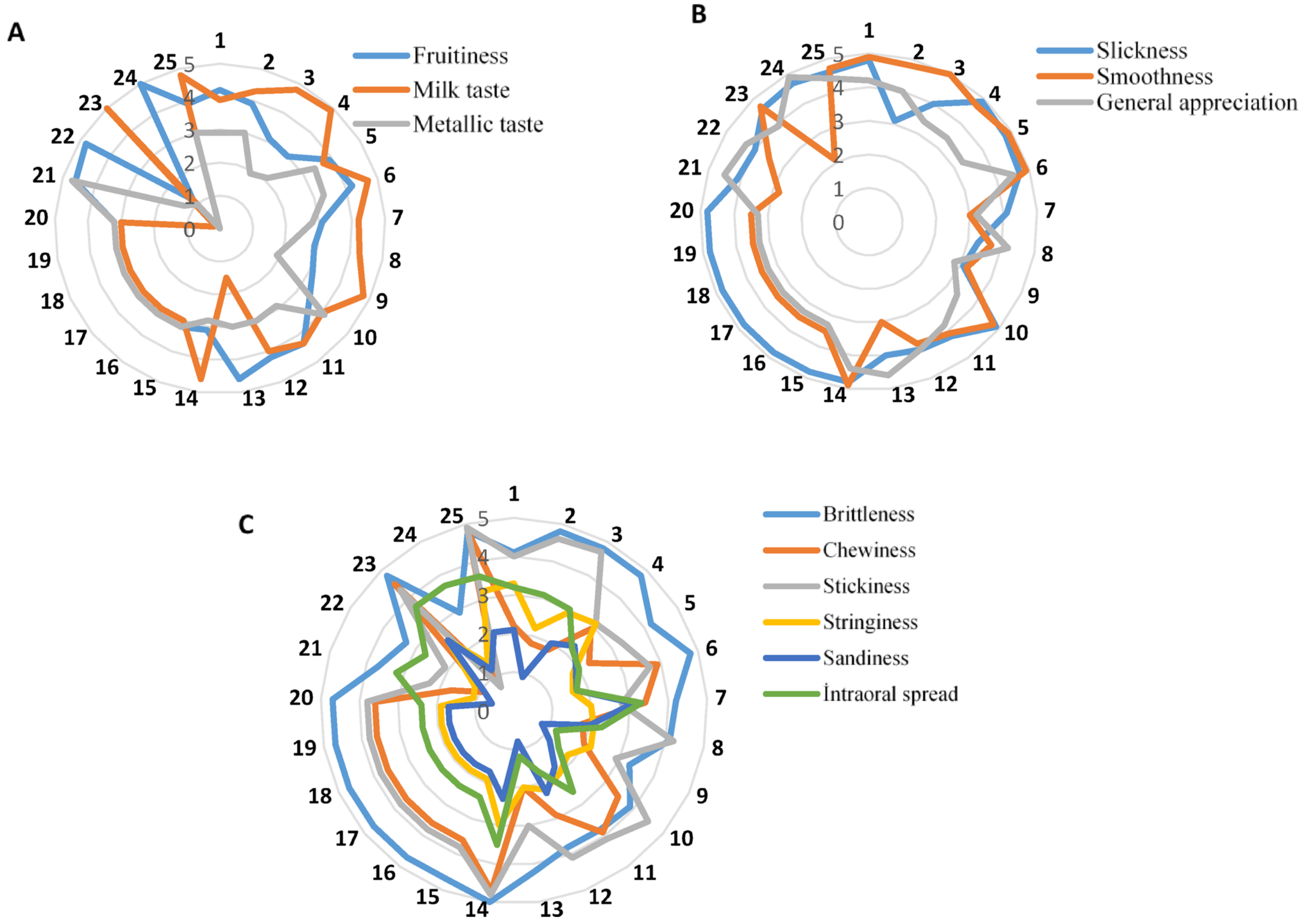
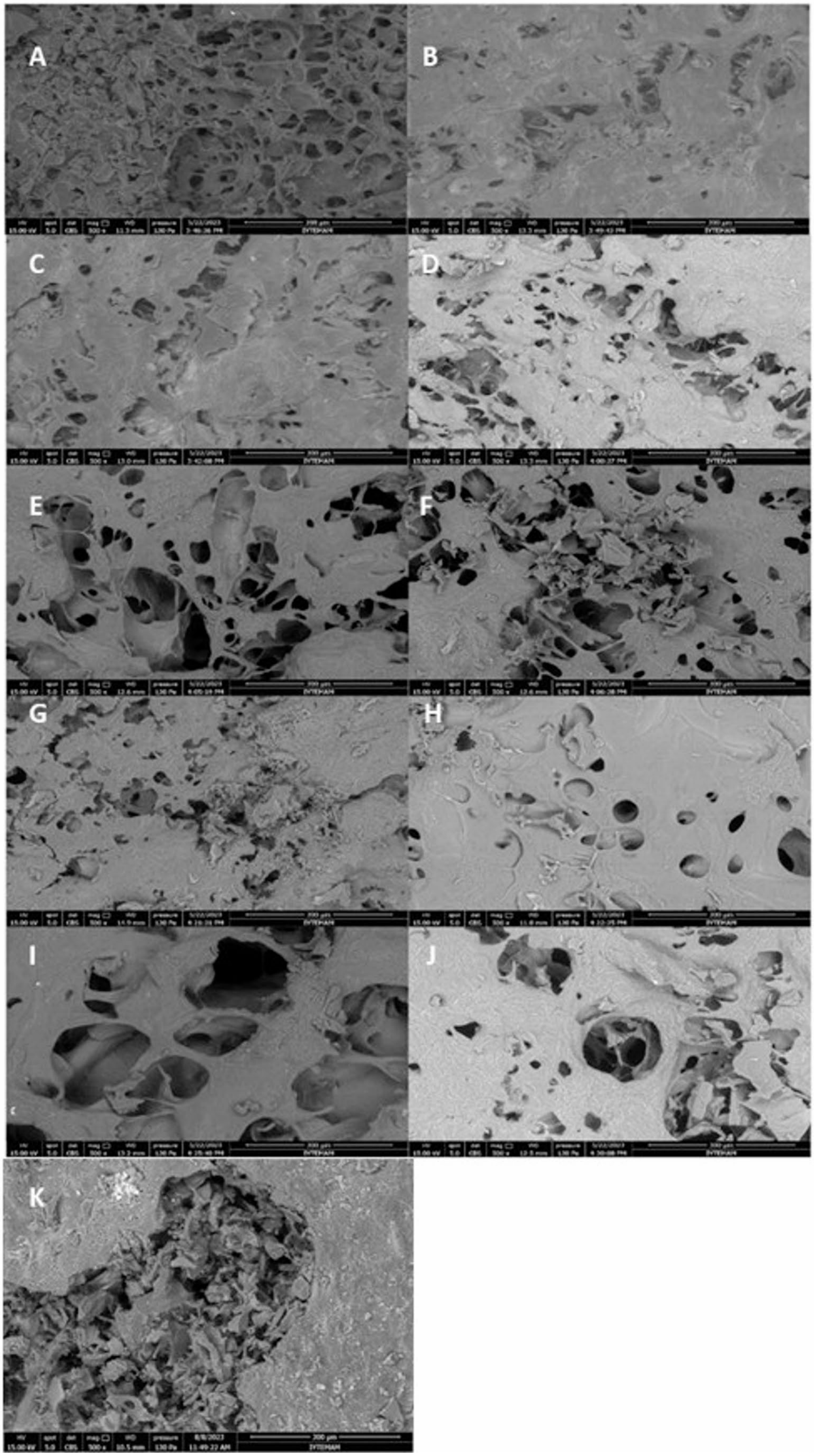


Fig. 2 The radar diagrams of the sensory analysis data for **A** the taste properties, **B** the appearance properties, **C** the texture properties


A
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◀ **Fig. 3** View of the cross-section of freeze-dried mixtures under scanning electron microscopy. **A** sample 1, 300 µm, **B** sample 2, 300 µm, **C** sample 6, 300 µm, **D** sample 8, 300 µm, **E** sample 12, 300 µm, **F** sample 13, 300 µm, **G** sample 14, 300 µm, **H** sample 21, 300 µm, **I** sample 22, 300 µm, **J** sample 24 (control), 300 µm, **K** sample 25 (optimum), 300 µm

of SA, and heterogeneous smaller pores became noticeable (Fig. 3B). This change slightly increased the drying efficiency. However, the fact that it also causes an increase in rehydration capacity suggests that the use of SA alone will not positively affect storage stability. Although the difference in the amount of gum arabic used did not affect the pore distribution, it caused an increase in the diameter and number of pore (Fig. 3C and D). This microstructural change caused a decrease in fracturability and hardness (Table 4). Due to the decrease in the amount of WPI, heterogeneity of the pore diameter increased as well as the number of larger pores decreased. It caused an increase in the fracturability of the structure and a decrease in hardness (Fig. 3E and F). While sample number 14, which contains high amounts of WPI, as well as GA and SA, had a fracture and low hardness structure; in sample coded 21, which contains only SA, fracturability decreased but hardness increased significantly compared to other samples (Table 4). In the microstructure images of the mentioned samples, it can be seen that sample coded 14 was heterogeneous and contains more pores (Fig. 3G); In the image of sample no. 21, it was observed that although the number of pores decreases considerably, the amorphous crystalline structure was evident (Fig. 3H). Large pores and homogeneous distribution were noteworthy in sample coded 22, which has the highest GA content at the concentration used in this research. The SEM image of the control sample showed small pores as well as the structure formed by the fibers of orange and persimmon with the formation of large heterogeneous pores (Fig. 3J). Although it has a low hardness value compared to other formulations, which is thought to be due to its natural fiber content, the reason for its low brittleness value is clearly observed. The sample with optimum component content gave a uniform appearance of crystallization characterized by the formation of an amorphous structure (Fig. 3K) which explained the reason for the high hardness value of this product (Table 4). However, at some points, large and small pores draw attention in the part that couldn't resist the enthalpy of evaporation (Fig. 3K). It is thought that high fracturability arised from areas where pores are abundant.

In the micrographs of the snacks coded 1 and 2, it was observed that increasing the amount of SA added by 3 times significantly reduced the porosity (Fig. 3A and B). Micrographs of snacks code 2 and 6 (Fig. 3B and C) reveal that increasing the amount of WPI added by 3 times reduced the porosity considerably. When the micrographs of sample coded 22 (Fig. 3I) and the control sample (Fig. 3J) were compared, it was determined that the GA added to the premixes did not cause a significant effect on the porosity structure of the snacks. The porosity status did not change in the samples with and without GA. When code 25 (optimum) (Fig. 3K) is compared with candy number 14 (Fig. 3G), which is closest to optimum in terms of content; differences in porosity were observed between micrographs. It is thought that the difference is related the amount of SA.

Conclusion

As public awareness of health benefits increases, consumers are seeking foods that support their well-being, and prompt food companies to develop functional products. The study focused on creating a snack that meets daily glutathione requirements using a nutrient-rich fruit blend. Among the additives tested, sodium alginate significantly improved the texture of freeze-dried snacks made from persimmon and orange puree, enhancing hardness and fracturability. SA not only boosted physical durability and consumer appeal but also improved storage stability by increasing the glass transition temperature more than other additives The benefits of SA were further amplified when combined with optimized ratios of SA, GA, and WPI. WPI effectively lowered water activity, contributing to better shelf life and stability. While SA had a minimal impact on water activity, further research with varying concentrations is needed. Although GA did not show a direct effect on water activity, it may yield valuable insights when tested with different formulations. The combined use of sodium alginate, gum arabic, and whey protein isolate in a nutrient-rich fruit-based snack formulation enhanced texture, stability, and shelf life, supporting its potential as a functional glutathione-rich product.

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Code availability Not applicable.

Declarations

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Consent to participate Authors declare that they have given their approval for all processes related to the publication of the manuscript.

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