Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 1, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

NEW CHOCOLATE FORMULATIONS WITH IMPROVED FUNCTIONALITY BY USING CAROB AND ROSEHIP POWDERS AS PARTIAL COCOA SUBSTITUTES

Ioana-Alina POP[1], Diana MOIGRADEAN[1], Daniela STOIN[1], Diana-Nicoleta RABA[2], Carmen Daniela PETCU[3], Delia-Gabriela DUMBRAVA[1], Mariana-Atena POIANA[1]

[1]University of Life Sciences "Regele Mihai I" from Timisoara, Faculty of Food Engineering, 119 Calea Aradului Street, 300645, Timisoara, Romania
[2]University of Life Sciences "Regele Mihai I" from Timisoara, Faculty of Management and Rural Turism, 119 Calea Aradului Street, 300645, Timisoara, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, District 5, 050097, Bucharest, Romania

Corresponding author email: marianapoiana@usvt.ro

Abstract

In this study, the bioactive potential of unconventional materials, such as carob powder (CP) and rosehip powder (RP), was exploited as partial substitutes for cocoa to design new chocolate formulations. Nine formulations were prepared under laboratory conditions by substituting cocoa (w/w), as follows: 0% (control sample), 10% CP, 20% CP, 30% CP, 40% CP, 30% CP and 10% RP, 20% CP and 20% RP, 10% CP and 30% RP, respectively, 40% RP. Changes in the proximate composition and bioactive profile of chocolate were assessed based on total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. Progressive increase in the level of CP led to improvements in the bioactive properties. The addition of CP and RP mixture resulted in a more pronounced boost in bioactive attributes with increasing RP level. The highest bioactive profile was achieved for the 40% RP formula. High levels of TPC and TFC strongly contributed to the improvement of chocolate's antioxidant activity. These findings recommend fortifying chocolate with phenolic compounds provided by CP and RP to extend the range of functional confectionary products.

Key words: *antioxidant activity, bioactive compounds, carob and rosehip powder, chocolate formulations.*

INTRODUCTION

In contemporary food technology, there is a growing emphasis on the development of functional foods that promote health and well-being. A key challenge in this area is formulating innovative chocolate products that enhance nutritional value while remaining economically viable for consumers (Salem & Fahad, 2012).

The increasing demand for food products with superior sensory and nutritional attributes has led to the investigation of novel ingredients. Food fortification serves two primary purposes: replenishing nutrients lost during processing and incorporating bioactive compounds to improve the functional properties of food. In particular, the addition of dried fruits as a source of antioxidants has been shown to enhance both the nutritional quality and health benefits of chocolate (Cagind & Ege, 2009).

Pseudo-fruits from the *Rosa* species, especially *Rosa canina* L., have long been utilized in both food and medicine due to their abundance of bioactive compounds, including vitamin C, phenolics, tannins, tocopherol, lycopene, lutein, zeaxanthin, and other carotenoids (Avallone et al., 1997; Turkben et al., 2010). Extensive research highlights the significant role of rosehip as a rich source of ascorbic acid and phenolic compounds. The ascorbic acid content in rosehip varies between 140 and 1100 mg/100 g, with *Rosa canina* L. exhibiting a comparatively lower concentration of 510 mg/100 g (Cui et al., 2014; Roman et al., 2013).

Both *in vivo* and *in vitro* studies have confirmed the anti-inflammatory and antioxidant properties of rosehip, attributed to its polyphenol, vitamin C, E and B content, as well as carotenoids, which may act synergistically (Papuc et al., 2023; Predescu et al., 2016). Furthermore, rosehip has demonstrated antimicrobial activity and has been associated with beneficial effects on chronic conditions such as osteoarthritis and rheumatoid arthritis. Additionally, rosehip exhibits anti-diabetic and anticancer properties (Marmol et al., 2017).

In light of its well-documented bioactivity, rosehips qualify as a nutritious food and potentional functional ingredient with significant health benefits (Negrean et al., 2024; Stryjecka et al., 2025).

Carob (*Ceratonia siliqua* L.) is a valuable plant source of natural sugars, with a high sucrose content (over 50%), together with dietary fiber, protein and essential minerals including calcium, potassium and phosphorus (Ozcan et al., 2007). It also contains significant levels of various polyphenolic compounds, including anthocyanidins, hydrolysable tannins, condensed tannins and flavan-3,4-diols (Petit & Pinilla, 1995).

As reported by Papagiannopoulos et al. (2004), carob pods contain extractable polyphenols amounting to 448 mg/kg, which include gallic acid (174 mg/kg), hydrolysable tannins (26 mg/kg), condensed tannins (15 mg/kg) and derivatives of myricetin (171 mg/kg), quercetin (53 mg/kg) and kaempferol (9 mg/kg). Furthermore, carob pulp provides a moderate protein content in the range of 3-4%, while maintaining a low fat concentration of about 0.6% (Avollone et al., 1997).

Widely recognized as a natural sweetener, carob possesses a flavor and color reminiscent of cocoa and is frequently used as a cocoa substitute in a variety of food products, including chocolate, confectionery, ice cream, beverages, and baked goods (Yousif & Alghzawi, 2000). Its cocoa-like aroma arises from the thermal degradation of sugars and the formation of odorant compounds through the Maillard reaction (Fadel et al., 2006). Carob powder, produced by roasting and grinding carob pods, is characterized by a low fat content (approximately 2.5%) and is an unconventional gluten-free material of vegetable origin suitable for the food industry.

In recent decades, due to their high nutritional value, carob and rosehip powders have attracted considerable research interest as functional food ingredients (Salem & Fahad, 2012). The partial substitution of cocoa with alternative ingredients in chocolate formulations offers advantages such as reduced fat content and the introduction of unique sensory and functional properties to the final product (Fadel et al., 2006).

Our preliminary results revealed the potential of carob and rosehip powders to improve chocolate quality (Moigradean et al., 2022). Based on these findings, the aim of this study was to develop chocolate formulations with high nutritional value and improved functionality by partially replacing cocoa powder with carob and rosehip powder, as well as with mixtures of carob and rosehip powder to enhance the content of high value bioactive phenolic compounds and antioxidant properties of the designed formulations.

MATERIALS AND METHODS

Raw materials

The ingredients involved in the production recipes such as refined sugar (Pfeifer & Langen GmbH & Co. KG, Koln, Germany), milk powder (Bartex SP. Z o.o., ul. Dworcow 4A, Paslek, Poland), butter with 80% fat (Friesland Campina Romania SA, Cluj-Napoca, Romania), cocoa powder (Barry Callebaut Cocoa AG, Zurich, Switzerland), carob powder (Sano Vita SRL, Vâlcea, Romania), rosehip powder (BioPlanet SA, Leszno, Poland), were purchased from local supermarkets and local specialty stores.

Chocolate production on a laboratory scale

The chocolate was prepared according to a traditional manufacture procedure applied on a small-scale level. The materials included in the recipe of chocolate formulations were reported in Table 1. It can be seen that in the control sample, 100 g of cocoa was used, and in the other formulations, cocoa powder was substituted with carob powder (CP), rosehip powder (RP) or mixture of CP and RP, respectively. The chocolate was prepared by mixing 500 g of sugar and 150 mL of water in a pot on the stove and bring the mixture to a boil until a drop taken between the fingers forms thin threads when separated. The boiling time varies depending on the heat intensity, but typically takes about 1-2 minutes from the moment boiling starts. Once this stage is reached, we removed the pot from the heat.

Table 1. Ingredients for preparing chocolate using carob powder and rosehip powder as partial cocoa substitutes

Ingredients	C	10CP	20CP	30CP	40CP	30CP10RP	20CP20RP	10CP30RP	40RP
Sugar (g)	500	500	500	500	500	500	500	500	500
Butter 80% fat (g)	100	100	100	100	100	100	100	100	100
Milk powder (g)	250	250	250	250	250	250	250	250	250
Cocoa (g)	100	90	80	70	60	60	60	60	60
Carob powder (g)	-	10	20	30	40	30	20	10	-
Rosehips powder (g)	-	-	-	-	-	10	20	30	40
Water (mL)	150	150	150	150	150	150	150	150	150

Next, the butter (100 g) was incorporated and allowed the mixture to cool slightly.

In a separate bowl, into which have previously weighed 250 g milk powder and 100 g cocoa powder, or cocoa powder substituted with CP and RP as given in Table 1, previously sifted through a fine sieve to ensure a uniform texture, add the butter and syrup mixture and mix vigorously until smooth.

The resulting mixtures are poured into silicone moulds and left to cool to room temperature, then refrigerated for about 4 hours.

The resulting products, shown in Figure 1, were labelled according to each formula as follows:

- C - control sample with 100% cocoa;
- 10CP - 10% of cocoa was replaced with CP;
- 20CP - 20% of cocoa was replaced with CP;
- 30CP - 30% of cocoa was replaced with CP;
- 40CP - 40% of cocoa was replaced with CP;
- 30CP10RP - 30% of cocoa powder was replaced with CP and 10% with RP;
- 20CP20RP - 20% of cocoa was replaced with CP and 20% with RP;
- 10CP30RP - 10% of cocoa was replaced with CP and 30% with RP;
- 40RP - 40% of cocoa was replaced with RP.

After being taken out of the refrigerator, the chocolates were removed from the moulds, and samples were taken from each formula, packed in polypropylene food storage bags, sealed and stored at −20°C until chemical analysis.

Proximate composition and energy value evaluation

The proximate composition of chocolate formulations was evaluated in accordance with the standard method described by the Association of Official Analytical Chemists (AOAC, 2000).

The carbohydrate content (%) was calculated by subtracting the protein, ash, lipid and moisture from 100. The energy value of the chocolate was calculated according to Das et al. (2019), taking into account that 1 g of carbohydrate contributes 4 calories, 1 g of fat contributes 9 calories and 1 g of protein contributes 4 calories, as shown in equation (1).

Energy value (kcal/100 g) = carbohydrates (%) x 4 + lipids (%) x 9 + proteins (%) x 4 (1)

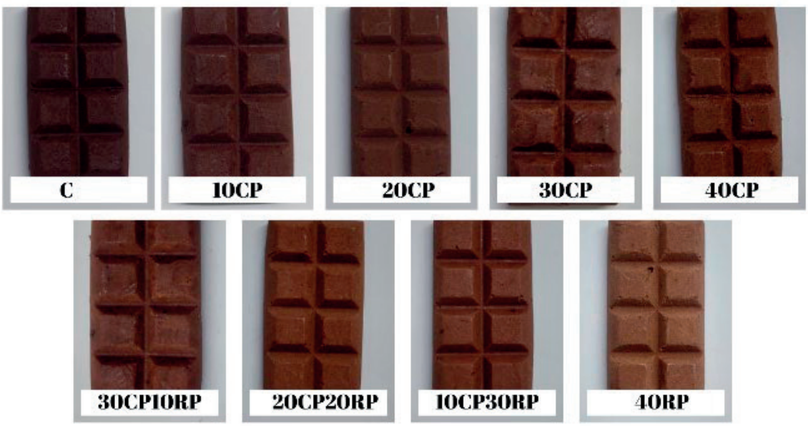

Figure 1. Chocolate formulas obtained by partially replacing cocoa powder with CP and RP (original)

Alcoholic extract preparation

The total phenolic content, total flavonoid content and antioxidant activity of the investigated samples were assessed in 70% (v/v) ethanol extracts, which were prepared according to the procedure described by Litwinek et al. (2023) and Metzner Ungureanu et al. (2020) with minor modifications. From CP, RP and defatted chocolate samples, 1.0 g was weighed into lidded containers and mixed with 10 mL of 70% (v/v) ethanol. The extraction was carried out for 2 h under continuous stirring at room temperature, and then, the mixtures were centrifuged at 10,000 rpm for 10 min (Hettich EBA 21 Centrifuge). The supernatant was collected and the residue was washed with 70% (v/v) ethanol and subjected to extraction for a further 1 h and then centrifuged in the same conditions. Afterwards, the supernatants were mixed and stored at -20°C until analysis.

Evaluation of total phenolic content (TPC)

TPC was determined by Folin-Ciocalteu method in agreement with the protocol described by Tolve et al. (2021) and Blanch et al. (2021). Dilution of the alcoholic extracts with distilled water in the following ratios 1:20 (v/v) for CP and RP and 1:10 (v/v) for chocolate samples, respectively, was carried out prior to analysis. The TPC was calculated using a standard gallic acid calibration curve in the concentration range 0.1-1.0 μM gallic acid equivalents (GAE)/mL. Results were expressed as mg GAE per 100 g of dry weight (d.s) of the sample.

Evaluation of total flavonoids content (TFC)

The total flavonoid content (TFC) was determined following the method outlined by Al-Farsi et al. (2018) with slight modifications. TFC was calculated using a standard quercetin (QE) calibration curve in the concentration range 0.5-50 μg/mL. The results were expressed as mg QE/100 g of dry weight (d.s).

Assessment of antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the samples was assessed using the DPPH assay, with a 0.1 mM DPPH solution prepared in 70% (v/v) ethanol in agreement with the procedure described by Antonic et al. (2021) and Poiana et al. (2023). The previously prepared ethanolic extracts have been further diluted with 70% (v/v) ethanol in ratios of 1:200 (v/v) for CP and RP and 1:10 (v/v) for chocolate formulations, respectively. The antioxidant activity was calculated and expressed as μM Trolox equivalent (TE)/g of dry weight (d.s) of the sample.

Assessment of antioxidant activity by ferric-reducing atioxidant power (FRAP) assay

The total antioxidant potential of the samples was evaluated using the ferric reducing antioxidant power (FRAP) assay following the procedure described by Alexa et al. (2012). Dilution of the extracts with distilled water prior to analysis was 1:200 (v/v) for CP and RP and 1:10 (v/v) for chocolate formulations, respectively. The results were expressed as μM Fe^{2+} equivalent per gram of dry weight (d.s) of the sample, using a standard $FeSO_4 \cdot 7H_2O$ calibration curve in the concentration range 0.05-0.5 μM Fe^{2+} equivalents per mL.

Statistical analysis

Each determination was performed in triplicate and results were reported as mean values ± standard deviation (SD). An analysis of variance (one-way ANOVA) was carried out to assess the statistical significance of the differences between the investigated samples.

RESULTS AND DISCUSSIONS

Analytical results for the proximate composition of chocolate formulations obtained by partial replacement of cocoa powder with carob powder (CP) and rosehip powder (RP) are presented in Table 2.

It can be seen that replacing cocoa powder with RP and CP in the chocolate recipe led to an increase in the moisture content of the samples. Compared to the control sample, CP fortified chocolate formulations showed an increase in moisture content from 1.66% in the 10CP formula to 8.11% in the 40CP formula.

Table 2. Proximate composition of chocolate formulations

Sample	Protein (%)	Lipids (%)	Ash (%)	Moisture content (%)	Carbohydrates (%)	Energy value (kcal/100 g)
C	8.37 ± 0.01^{a}	16.42 ± 0.02^{a}	1.91 ± 0.03^{a}	5.42 ± 0.03^{i}	67.88	452.78
10CP	8.21 ± 0.02^{b}	16.19 ± 0.03^{b}	1.84 ± 0.01^{b}	5.51 ± 0.02^{h}	68.25	451.55
20CP	8.06 ± 0.01^{c}	16.02 ± 0.04^{c}	1.76 ± 0.01^{c}	5.62 ± 0.01^{g}	68.54	450.58
30CP	7.92 ± 0.03^{d}	15.81 ± 0.03^{d}	1.68 ± 0.02^{d}	5.75 ± 0.02^{f}	68.84	449.33
40CP	7.79 ± 0.01^{e}	15.60 ± 0.02^{e}	1.61 ± 0.01^{e}	5.86 ± 0.01^{e}	69.14	448.12
30CP10RP	7.71 ± 0.01^{e}	15.66 ± 0.04^{e}	1.66 ± 0.02^{d}	5.92 ± 0.02^{d}	69.05	447.98
20CP20RP	7.65 ± 0.02^{c}	15.73 ± 0.03^{c}	1.72 ± 0.02^{d}	6.03 ± 0.03^{c}	68.87	447.65
10CP30RP	7.58 ± 0.01^{d}	15.81 ± 0.02^{d}	1.77 ± 0.01^{c}	6.12 ± 0.02^{b}	68.72	445.09
40RP	7.49 ± 0.03^{b}	15.87 ± 0.05^{b}	1.83 ± 0.02^{b}	6.25 ± 0.04^{a}	68.56	447.03

Values are reported as the mean of three independent analyses ± standard deviation (SD). Values in a column having different superscripts are statistically different (one-way ANOVA, $p < 0.05$).

The moisture content of the RP-added chocolate samples was much higher than that of the control sample.

Replacing cocoa powder with a mixture of CP and RP powder increased the moisture content of the samples, relative to the control, by 9.22% for 30CP10RP, 11.25% for 20CP20RP and 12.91% for the 10CP30RP formulation, respectively.

In addition, replacing the cocoa powder with 40% RP resulted in the highest moisture content, resulting in an increase of 18.36% over the control sample. Therefore, a significant ($p < 0.05$) increase in the moisture content was observed in the samples with the highest amount of RP used to replace cocoa.

The data in Table 2 also shows that the protein content decreases in samples where cocoa powder was replaced by CP and RP.

The induced changes in protein content can be observed with a gradual decrease from 8.37% in control sample to 7.79% in the 40CP formula.

RP fortified samples also decrease slowly in protein content, ranging from 7.71% in 30CP10RP to 7.49% in 40RP.

The CP and RP formulations have slightly higher carbohydrate content than the control, but the differences are not significant.

The increase ranges from 0.37%, relative to the control sample in the 10CP formula, to 0.68% in the 40RP sample compared to the control, while the ash, protein and lipid contents decrease slightly.

This finding is closely related to the contribution made by CP and RP addition and has been also reported in a study on chocolate formulation with carob addition by Lagha-Benamrouche & Hezil (2023) who reported that CP enriched chocolates were slightly lower in fat and protein.

The fat content of chocolate formulations decreases slightly with substitution of cocoa powder with RP and CP.

In chocolate formulations for which cocoa was replaced by CP, the decrease in lipid content compared to the control sample ranged from 1.40% for 10CP to 4.99% for 40CP formula.

In the formulations where RP was added as a partial substitute for cocoa powder there was also a decrease in fat content compared to the control sample. However, the decrease is slightly less than in the samples where only CP was added as a cocoa replacer.

For the 30CP10RP sample a decrease of 4.62% compared to the control sample was noted, while for the 40RP sample the fat content decreased by 3.34% compared to the control sample.

The content of ash in CP chocolates was 1.91 g/100 g (control) and it very slowly decreased, to 1.61 g/100 g (40CP). In chocolates with RP, the ash content slightly increases from those with only CP, from 1.66 g/100 g in the sample 30CP10RP to 1.83 g/100 g in the 40% RP sample (40RP), but the ash content are still slightly lower than those of the C sample.

The energy value was not strongly influenced by substitution of cocoa with CP and RP decreases from 452.78 kcal/100 g in control sample, to 448.12 kcal/100 g in 40CP and to 447.03 kcal/100 g in 40RP chocolate formula.

The total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity evaluated by FRAP and DPPH assays were tested in this study for CP and RP, and the results are shown in Table 3.

Table 3. Total phenolic content and total flavonoids content along with antioxidant activity of CP and RP

Phytochemicals and antioxidant activity	CP	RP
TP (mg GAE/100 g d.s)	1686.64 ± 1.71[b]	2307.59 ± 1.89[a]
TF (mg QE/100 g d.s)	1108 ± 1.69[b]	1408.28 ± 1.81[a]
FRAP (μM Fe^{2+}/g d.s)	398.25± 1.29[b]	517.54 ± 1.48[a]
DPPH (μM TE/g d.s)	466.45 ± 1.37[b]	590.54 ± 1.52[a]

Values reported are the mean of three independent analyses ± standard deviation (SD). Values in a row having different superscripts are statistically different (one-way ANOVA, $p < 0.05$).

The most important class of bioactive substances found in CP and RP are the phenolic compounds. The antioxidant activity of rosehip is attributed to its content of polyphenols, vitamins C, E, B and carotenoids, which may exhibit synergistic effects (Marmol et al., 2017).

The data shown in Table 3 indicates that TPC of CP is 1686.64 mg GAE/100 g d.s, consistent with the findings of Ioannou et al. (2023) Information on the total phenolic content of carob pods is limited in the literature. However, it has been shown to vary depending on several factors, including the extraction method, geographical origin, variety, cultivation conditions, and maturity level (Atalanti et al., 2021).

The TPC of RP resulted was 2307.59 mg GAE/100 g d.s, similar to the findings of Igual et al. (2022) who showed results of 2482 mg GAE/100 g d.s, proving the RP has an even higher phenolic content that CP, which is consistent with the literature found in this domain.

The flavonoid content of CP (1108.0 mg QE/100 g d.s) was slightly lower than that of RP (1408.28 mg QE/100 g d.s).

In regards to the antioxidant activity of CP, a value of 398.25 µM Fe^{2+}/g d.s by FRAP assay and 466.45 µM TE/g DW by DPPH assay was obtained, while for RP, the results were 517.54 µM Fe^{2+}/g d.s by FRAP assay and 590.54 µM TE/g DW by DPPH assay.

The data in Table 3 reflect significantly ($p<0.05$) higher values of TPC, TFC, FRAP and DPPH for RP compared to CP.

The functional potential of CP and RP has been further exploited in chocolate formulations prepared according to a simple recipe commonly used at the household level. This approach integrates raw material requirements into the assessment of functional properties of traditional chocolate products.

Tests were performed to find out the changes in phytochemical content and antioxidant activity of chocolate by progressively replacing cocoa powder from 10 to 40% with CP. Then, in 40CP formulation, CP was replaced with RP from 10 to 40%.

The total phenolic content and total flavonoid content of developed chocolate formulas are shown in Table 4.

Table 4. Changes in total phenolic content and total flavonoid content of chocolate formulas

Chocolate sample	TPC (mg GAE/100 g d.s)	TFC (mg QE/100 g d.s)
C	454.32 ± 1.19[i]	175.39 ± 1.05[i]
10CP	471.09 ± 1.23[h]	187.03 ± 1.1 [h]
20CP	498.25 ± 1.32[g]	203.16 ± 1.31[g]
30CP	526.95 ± 1.28[f]	219.42 ± 1.15[f]
40CP	554.53 ± 1.25[e]	231.23 ± 1.34[e]
30CP10RP	579.42 ± 1.34[d]	240.49 ± 1.18[d]
20CP20RP	597.13 ± 1.18[c]	251.13 ± 1.22[c]
10CP30RP	627.90 ± 1.37[b]	263.04 ± 1.29[b]
40RP	651.31 ± 1.41[a]	272.8 6 ± 1.33[a]

Values reported are the mean of three independent analyses ± standard deviation (SD). Values in a column having different superscripts are statistically different (one-way ANOVA, $p < 0.05$).

It can be seen that using CP and RP as substitutes for cocoa in different concentrations in the enriched chocolate formulations led to a progressive augmentation of TPC.

The highest value of TPC was found in the sample with 40% RP, where TPC increased by 43.35% compared to the control sample.

In the formulations of chocolate with only CP as a cocoa substitute, the increase is lower than in those with RP, but still significant, ranging from 3.69% increase from C in the 10CP sample, to 22.05% increase in the 40CP sample. The TPC increased from 454.32 mg GAE/100 g d.s in control sample to 554.53 mg GAE/100 g d.s in 40CP to 651.31 mg GAE/100 g d.s in the 40RP sample.

The total flavonoid content (TFC) also increased significantly in the samples with CP and RP. Substituting cocoa with CP increased TFC by 6.63% in the 10CP sample, to 31.83% in the 40CP sample, compared to C.

While the control sample showed TFC to be 175.39 mg QE/100 g d.s, the 10CP sample showed 187.03 mg QE/100 g d.s, and the maximum CP addition as substitute (40CP) resulted in 231.23 mg QE/100 g d.s. The highest TFC content was registered in the formulations with RP, where the TFC increase reached 55.57% in 40RP from C, more exact, a content of 272.86 mg QE/100 g d.s.

The antioxidant activity expressed by FRAP value of the chocolate formulas are shown in Figure 2.

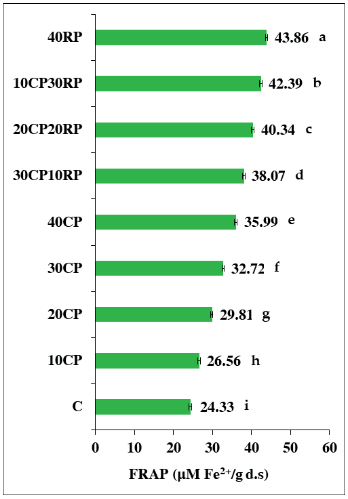

Figure 2. Changes in FRAP value of chocolate formulas. Values are the mean of three independent analyses ± SD. Values for bars with different letters are statistically different (one-way ANOVA, $p < 0.05$).

Substituting cocoa powder with CP and RP increased the antioxidant activity significantly.

Chocolate formulations that included different incorporation levels of CP and RP were found to be significantly higher (p<0.05) in both FRAP and DPPH values over the control.

The FRAP value increases achieved in chocolates with CP increases with 9.16% in the 10CP sample, to 47.92% in the 40CP formulation, when compared to control. The chocolates with RP the FRAP value increases significantly, from 56.47% over the C sample in the 30CP10RP formulation, to 80.27% in the formulation with 40% cocoa substituted by rosehip powder (RP).

The samples with the most RP showed the highest antioxidant activity due to the high level of bioactive compounds of RP. The FRAP value of control resulted in 24.33 μM Fe^{2+}/g d.s. The results of 40CP showed 35.99 μM Fe^{2+}/g d.s, while the highest FRAP vale was for the 40RP sample, respectively 43.86 μM Fe^{2+}/g d.s.

The antioxidant activity expressed by DPPH value of the chocolate formulas are illustrated in Figure 3.

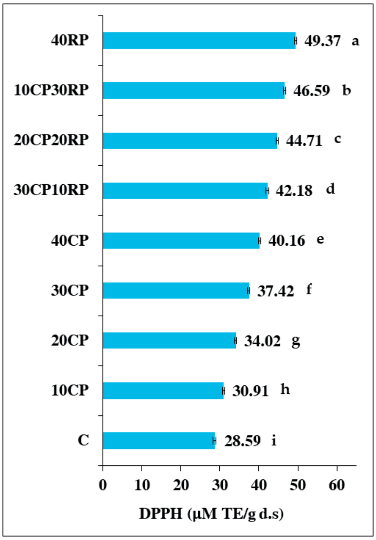

Figure 3. Changes in DPPH value of chocolate formulas. Values are the mean of three independent analyses ± SD. Values for bars with different letters are statistically different (one-way ANOVA, $p < 0.05$)

DPPH value also increases with addition of CP and RP as a cocoa substitute. Our results clearly show the significant increase of DPPH in formulations where cocoa is substituted, as follows: the 10CP sample shows an increase of 8.11% compared to control, while the 40% CP as cocoa substitute, increases DPPH by 40.46% compared to control sample.

As with the FRAP value, the DPPH value is highest when RP is added as a substitute for cocoa, with increases from 47.53% in the 30CP10RP sample to 70.68% in the 40% RP formulation, compared to the control. Therefore, substitution of cocoa powder in chocolate manufacturing with CP and RP should improve functional properties and nutritional values of products.

CONCLUSIONS

This study demonstrates that the incorporation of CP into chocolate significantly improves the total polyphenolic compounds and total flavonoids content. In addition, PC provides a viable alternative to cocoa powder in the chocolate production.

The results show a tight correlation between the concentration of bioactive compounds and the proportion of CP added.

Chocolate samples fortified with both CP and RP showed significantly higher levels of antioxidant compounds compared to the control sample.

Due to their notable antioxidant activity, expressed both as ferric reducing antioxidant power and free radical scavenging activity, RP and CP are valuable bioactive ingredients for the development of chocolate formulations with improved functional properties.

On the basis of these findings, CP and RP are recommended as effective agents to improve the antioxidant profile and the content of bioactive compounds in chocolate.

Furthermore, the partial replacement of cocoa powder by CP and RP contributes to the diversification of chocolate varieties available on the market.

Chocolate is a complex matrix composed of several ingredients and changes in its formulation may influence the stability and preservation of antioxidants naturally present in the raw materials. The addition of CP and RP represents a practical and sustainable option for the chocolate industry.

Although RP and CP fortified chocolate shows promise as a functional food, further research is needed to fully explore its potential benefits.

REFERENCES

Alexa, E., Poiana, M.A. & Sumalan, R.M. (2008) Mycoflora and Ochratoxin A control in wheat grain using natural extracts obtained from wine industry by-products. *International Journal of Molecular Sciences, 13*, 4949–4967. https://doi.org/10.3390/ijms13044949

Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A., & Al-Belushi, S. (2018). Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon, 4*, e00874. https://doi.org/10.1016/j.heliyon.2018.e00874

Antonic, B., Dordevic, D., Jancikova, S., Holeckova, D., Tremlova, B. & Kulawik, P. (2021). Effect of grape seed flour on the antioxidant profile, textural and sensory properties of waffles. *Processes*, *9*(1), 131. https://doi.org/10.3390/pr9010131

Atalanti, C., Stavrou, I. J., & Kapnissi-Christodoulou, C. P. (2021). Continuous and pulsed ultrasound-assisted extraction of carob's antioxidants: Processing parameters optimization and identification of polyphenolic composition. *Ultrasonics Sonochemistry, 76*, 105630. https://doi.org/10.1016/j.ultsonch.2021.105630

Association of Official Analytical Chemists (AOAC). (2000). *Official methods of analysis* (17th ed.). Washington, DC, USA: AOAC Publishing House.

Avollone, R., Plessi, M., Baraldi, M., & Monzani, A. (1997). Determination of chemical composition of carob (*Ceratonia siliqua*): Protein, fat, carbohydrates, and tannin. *Journal of Food Composition and Analysis, 1*, 166–172.

Blanch, G. P., & Ruiz del Castillo, M. L. (2021). Effect of baking temperature on the phenolic content and antioxidant activity of black corn (*Zea mays L.*) bread. *Foods, 10*, 1202.

Cagınd, O., & Ege, S. O. (2009). The health benefits of chocolate enrichment with dried fruits. *Scientiarum Polonorum Technologia Alimentaria, 8*(4), 63–69.

Cui, F., Pacier, C., & Martirosyan, M. D. (2014). Rose hip (*Rosa canina L*): A functional food perspective. *Functional Foods in Health and Disease, 4*(12), 493–509.

Das, P. C., Khan, M. J., Rahman, M. S., Majumder, S., & Islam, M. N. (2019). Comparison of the physico-chemical and functional properties of mango kernel flour with wheat flour and development of mango kernel flour based composite cakes. *NFS Journal, 17*, 1–7.

Fadel, H. H. M., Abdel Mageed, M. A., Abdel Samad, A. K. M. E., & Lotfy, S. N. (2006). Cocoa substitute: Evaluation of sensory qualities and flavor stability. *European Food Research and Technology, 223*(1), 125–131.

Igual, M., García-Herrera, P., Cámara, R. M., Martínez-Monzó, J., García-Segovia, P., & Cámara, M. (2022). Bioactive compounds in rosehip (*Rosa canina*) powder with encapsulating agents. *Molecules, 27*(15), 4737. https://doi.org/10.3390/molecules27154737

Ioannou, G. D., Savva, I. K., Christou, A., Stavrou, I. J., & Kapnissi-Christodoulou, C. P. (2023). Phenolic profile, antioxidant activity, and chemometric classification of carob pulp and products. *Molecules, 28*(5), 2269. https://doi.org/10.3390/molecules28052269

Lagha-Benamrouche, S., & Hezil, D. (2023). Production of chocolate enriched with carob powder. *Revue Agrobiologia, 13*(2), 3597–3605.

Litwinek, D., Gumul, D., Łukasiewicz, M., Zi˛eba, T., & Kowalski, S. (2023). The effect of red potato pulp preparation and stage of its incorporation into sourdough or dough on the quality and health-promoting value of bread. *Applied Sciences, 13*, 7670.

Marmol, I., Sanchez-de-Diego, C., Jimenez-Moreno, N., Ancin-Azpilicueta, C., & Rodriguez-Yoldi, M. J. (2017). Therapeutic applications of rose hips from different *Rosa* species. *International Journal of Molecular Sciences, 18*(6), 1137.

Metzner Ungureanu, C.-R., Lupitu, A.I., Moisa, C., Rivis, A., Copolovici, L.O., & Poiana, M.-A. (2020). Investigation on high-value bioactive compounds and antioxidant properties of blackberries and their fractions obtained by home-scale juice processing. *Sustainability, 12*, 5681. https://doi.org/10.3390/su12145681

Moigradean, D., Poiana, M. A., Gruiescu, R. D., Alda, L. M., & Bordean, D. M. (2022). Developing innovative value-added chocolate formulas by rosehip and carob powder incorporation. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management (SGEM), 22*(6.2), 135–141. https://doi.org/10.5593/sgem2022v/6.2/s25.18

Negrean, O.-R., Farcas, A.C., Nemes, S.A., Cic, D.-E., & Socaci, S.A. (2024). Recent Advances and Insights into the Bioactive Properties and Applications of *Rosa canina* L. and Its By-products. *Heliyon*, *10*, e30816. https://doi.org/10.1016/j.heliyon.2024.e30816

Ozcan, M. M., Arslan, D., & Gokcalik, H. (2007). Some compositional properties and mineral contents of carob (*Ceratonia siliqua*) fruit, flour, and syrup. *International Journal of Food Sciences and Nutrition, 58*(8), 652–658.

Papagiannopoulos, M., Wollseifen, H. R., Mellenthin, A., Haber, B., & Galensa, R. (2004). Flavonoid composition and antioxidant activity of carob pod extracts. *Journal of Agricultural and Food Chemistry, 52*, 3784–3791.

Papuc, C., Nicorescu, V., Predescu, N.C., & Petcu, C.D. (2013). Antioxidant Activity of Polyphenols Extracted from Dog Rose (Rosa canina) Fruits on Myoglobin and Lipids in Refrigerated Minced Beef. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine, *70*(1).

Petit, M. D., & Pinilla, J. M. (1995). Production and purification of a sugar syrup from carob pods. *LWT - Food Science and Technology, 28*(1), 145–152. https://doi.org/10.1016/S0023-6438(95)80027-1

Poiana, M. A., Alexa, E., Radulov, I., Raba, D. N., Cocan, I., Negrea, M., Misca, C. D., Dragomir, C., Dossa, S., & Suster, G. (2023). Strategies to formulate value-added pastry products from composite flours based on spelt flour and grape pomace powder. *Foods, 12*, 3239. https://doi.org/10.3390/foods12173239

Predescu, N. C., Papuc, C., Nicorescu, V., Gajaila, I., Goran, G. V., Petcu, C, D., Stefan, G. (2016). The influence of solid-to-solvent ratio and extraction method on total phenolic content, flavonoid content and antioxidant properties of some ethanolic plant extracts. *Rev. Chim., 67*(10), 1922–1927.

Roman, I., Stanila, A., & Stanila, S. (2013). Bioactive compounds and antioxidant activity of *Rosa canina L.* biotypes from spontaneous flora of Transylvania. *Chemistry Central Journal, 7*(1), 73.

Salem, E. M., & Fahad, A. O. (2012). Substituting of cacao by carob pod powder in milk chocolate manufacturing. *Australian Journal of Basic and Applied Sciences, 6*(3), 572–578.

Stryjecka, M., Kiełtyka-Dadasiewicz, A., Michalak, M. (2025). Physico-Chemical Characteristics of *Rosa canina* L. Seeds and Determining Their Potential Use. *Applied Sciences*, *15*, 168. https://doi.org/10.3390/app15010168

Tolve, R., Simonato, B., Rainero, G., Bianchi, F., Rizzi, C., Cervini, M., & Giuberti, G. (2021). Wheat bread fortification by grape pomace powder: Nutritional, technological, antioxidant, and sensory properties. *Foods, 10*, 75.

Turkben, C., Uylaser, V., Incedayi, B., & Celikkol, I. (2010). Effects of different maturity periods and processes on nutritional components of rosehip (*Rosa canina* L.). *Journal of Food Agricultural Environment, 8*, 26–30.

Yousif, A. K., & Alghzawi, H. M. (2000). Processing and characterization of carob powder. *Food Chemistry, 69*(3), 283–287.