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# Citation

MRÁZKOVÁ, Martina, Daniela SUMCZYNSKI, and Jana ORSAVOVÁ. Non-traditional muesli mixtures supplemented by edible flowers: Analysis of nutritional composition, phenolic acids, flavonoids and anthocyanins. *Plant Foods for Human Nutrition* [online]. vol. 76, iss. 3, Springer, 2021, p. 371 - 376 [cit. 2023-02-06]. ISSN 0921-9668. Available at

https://link.springer.com/article/10.1007/s11130-021-00918-3

# DOL

https://doi.org/10.1007/s11130-021-00918-3

# Permanent link

https://publikace.k.utb.cz/handle/10563/1010531

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# Non-Traditional Muesli Mixtures Supplemented by Edible Flowers: Analysis of Nutritional Composition, Phenolic acids, Flavonoids and Anthocyanins

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#### **Abstract**

In this study, the effect of using medicinal plants on nutrition composition and biologically active substances in cereal mixtures were investigated. In order to develop new type of non-traditional muesli mixtures supplemented with edible flowers, eight muesli mixtures were prepared applying the mixing ratio of 60—70% of non-traditional flakes and 30—40% of lyophi-lized fruits and edible flowers. This study examines nutritional composition, digestibility, fibres and phenolics of nutraceutical muesli mixtures using enzymatic-gravimetric and chromatographic methods. It shows that the mixture of kamut, einkorn, red and black quinoa or rice flakes together with hibiscus, mallow, rose, fruits has increased the fibre content (11.9—21.2%) and in vitro digestibility (87.8—93.8%). The greatest concentrations of individual phenolic contents were determined in free and soluble bound fractions of the mixtures. Cyanidin-3-glucoside (up to 116 mg/kg) and delphinidin-3-glucoside (up to 76.9 mg/kg) were established as major anthocyanins. Considering the individual phenolic fractions, sinapic and proto-catechuic acids were the most abundant phenolic acids and quercetin and epigallocatechin represented the most frequent flavonoids. These results indicate that non-traditional muesli with edible flowers containing a high amount of nutrients and bioactive substances have the potential to enhance a nutritionally balanced diet.

Keywords: Edible flower, In vitro digestibility, Muesli, Non-traditional flake, Phenolics

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#### Introduction

The updated scientific studies specify that diets high in whole grains and fruits are widely recommended for their health-promoting properties. Whole grains and fruits have historically held a place in dietary guidance because they provide many significant nutrients (proteins, fibres, vitamins) and phytochemicals (anthocyanins, phenolic acids) exhibiting biological activity [1-3]. Studies have signified that the consumption of whole grains and fruits decreases a risk of cardiovascular diseases, cancer and inflammation [4]. The soluble and insoluble bound phenolics may be released at variable gastrointestinal conditions and under colonic fermentation and may impart health benefits even at the local sites such as intestinal epithelium and beyond after absorption [5]. According to the legislation, a muesli mixture is defined as a mixture of milled cereal products modified by flocculation, extrusion, or another advanced technology. Further components may be added to the mixture as well; e.g., dried nuts, seeds, processed fruits or substances modifying taste, odour, or consistency [6]. Since diseases of civilization occur more, aromatic medicinal plant ingredients have attracted more consumers' attention, including edible flowers as a new ingredient. Edible flowers (lavender, rose, hibiscus, mallow, etc.) have comparable contents of phytochemicals with fruits, which made them possible to be introduced them among nutraceuticals [7, 8]. A lot of industrial application of edible flowers focus on flower tea, jelly, salads, herbal infused drinks, candied flowers, juice production and gastronomic meals [7], whereas application in combination with cereals and fruits has been neglected yet. Therefore, the hypothesis of adding the edible flower omponents to the mixture in order to increase nutritional value was promised [9].

The objective of this study was to prepare and analyse nontraditional muesli mixtures supplemented with edible flowers. Firstly, this study was undertaken to investigate some basic chemical characteristics (dry matter, ash, fat, starch and crude protein contents) and to determine insoluble dietary and neutral-detergent fibre contents. Moreover, dry and organic matter in vitro digestibility values were assessed using pepsin and pancreatin digestion. The individual profiles of selected phenolics in free, soluble and insoluble bound fractions and anthocyanins were determined using HPLC-DAD methods.

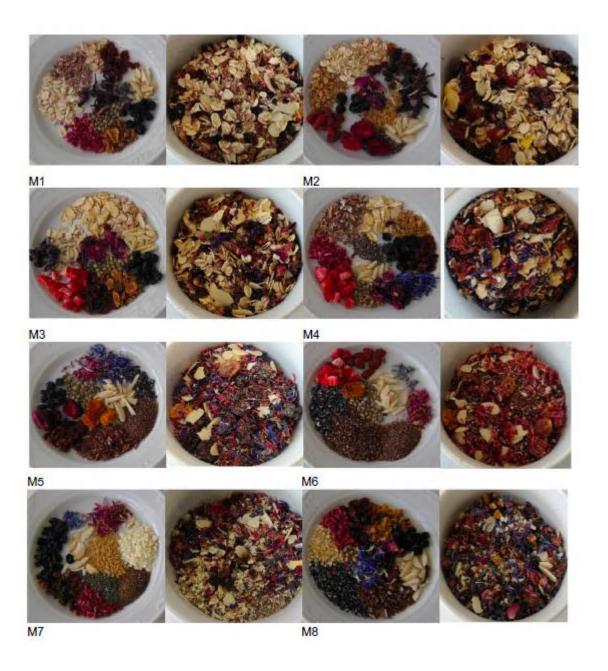
# **Materials and Methods**

The material and methods section is presented as **supplementary material**.

#### **Results and Discussion**

### **Basic Chemical Parameters and Digestibility Assay**

The detailed specific composition of the proposed muesli mixtures is provided in **Table S1** and **Fig. 1**. As can be seen in **Table 1**, the amount of dry matter ranged between 90.5 and 91.3%. According to the regulation [6], the moisture content of cereal products should not exceed 14%. The ash contents fluctuated between 2.02-2.80%. The highest value was registered in gluten-free M6 sample. Data dealing with ash content in commercial muesli mixtures has been scarce, but it is usually below 1.6% [10]. Such a relatively high level of ash shows that muesli mixtures might serve as a decent quality source of minerals. Protein contents in the mixtures varied from 12.3 to 15.5%. These nutrition values are comparable to the values measured by Kobus-Cisowska et al. [11].



**Fig. 1** Non-traditional muesli mixtures supplemented by non-traditional flakes and edible flowers according to recipe presented in Table S1. M1-M4 List of muesli mixtures containing gluten, M5-M8 List of gluten-free muesli mixtures

Table 1 Results of basic chemical analyses, fibre contents and in vitro digestibility assessments

Values (%)	Dry matter	Ash	Crude protein	Crude fat	Starch	IDF	NDF	DMD	OMD
M1	91.0±0.2 <sup>a,b,A</sup>	2.27 ± 0.01 <sup>a,A</sup>	12.3 ± 0.2a,A	7.29 ± 0.20 <sup>a,A</sup>	44.0±0.4 <sup>a,A</sup>	1.31 ± 0.02 <sup>a,A</sup>	21.2 ± 2.0 <sup>a,A</sup>	88.1 ± 1.0 <sup>a,A</sup>	92.9±0.7ª,A
M2	$90.9 \pm 0.1^{a,A}$	$2.38 \pm 0.10^{\mathrm{b,B}}$	$13.9 \pm 0.3^{b,B}$	$8.78 \pm 0.10^{\mathrm{b,B}}$	$43.7 \pm 0.5^{a,e,A}$	$1.33 \pm 0.01^{a,A}$	$20.3 \pm 0.2^{b,B}$	$89.1 \pm 1.1^{b,B}$	$93.2 \pm 1.0^{b,B}$
M3	$91.0 \pm 0.3^{a,b,A}$	$2.19 \pm 0.10^{c,C}$	$12.6 \pm 0.3^{a,d,A}$	$7.24 \pm 0.20^{a,A}$	$45.6 \pm 0.4^{b,B}$	$1.42 \pm 0.02^{b,B}$	$16.5 \pm 0.5^{c,C}$	$89.2 \pm 0.9^{b,B}$	$93.5 \pm 0.9^{b,B}$
M4	$90.8 \pm 0.2^{a,A}$	$2.52 \pm 0.10^{\rm d,D}$	$14.4 \pm 0.3^{c,C}$	$6.36 \pm 0.20^{c,C}$	$50.0 \pm 0.5^{c,C}$	$1.42 \pm 0.03^{b,B}$	$20.0 \pm 0.5^{a,A}$	$87.4 \pm 0.4^{c,C}$	$92.3 \pm 0.4^{a,A}$
M5	$91.1 \pm 0.2^{b,d,A,C}$	$2.39 \pm 0.10^{b,A}$	$12.7 \pm 0.2^{d,f,A}$	$7.71 \pm 0.20^{d,A}$	$42.6 \pm 0.5^{d,A}$	$1.41 \pm 0.01^{b,A}$	$17.8 \pm 1.0^{ m d,A}$	$80.9 \pm 1.2^{d,A}$	$87.8 \pm 1.4^{c,A}$
M6	$90.8 \pm 0.2^{a,A}$	$2.80 \pm 0.12^{e,B}$	$13.6 \pm 0.1^{b,B}$	$7.55 \pm 0.20^{e,B}$	$43.4 \pm 0.5^{\mathrm{e,B}}$	$1.54 \pm 0.01^{c,B}$	$14.7 \pm 0.6^{e,B}$	$81.5 \pm 0.4^{e,B}$	$89.1 \pm 0.6^{d,B}$
M7	$90.5 \pm 0.1^{c,B}$	$2.18 \pm 0.10^{c,C}$	$15.5 \pm 0.3^{e,C}$	$8.31 \pm 0.20^{f,C}$	$43.7 \pm 0.4^{a,e,B}$	$1.54 \pm 0.01^{c,B}$	$16.8 \pm 1.0^{c,C}$	$82.8 \pm 1.3^{f,C}$	$89.7 \pm 1.0^{d,B}$
M8	$91.3 \pm 0.1^{d,C}$	$2.02 \pm 0.10^{\rm f,D}$	$12.9 \pm 0.1^{f,C}$	$8.09 \pm 0.20~{\rm g,D}$	$42.8 \pm 0.4^{\rm d,A}$	$1.39 \pm 0.01^{d,A}$	$11.9\pm1.0^{\rm f,D}$	$89.8 \pm 0.7^{b,D}$	$93.8 \pm 0.6^{b,C}$

Results are presented on dry weight basis as means  $\pm$  SD (n=5). Means within a column with at least one identical small superscript (in case of all samples) do not differ significantly (P>0.05), means within a column with at least one identical capitalized superscript (in case of gluten-free and gluten containing mixtures) do not differ significantly (P>0.05), according to Tukey's test. IDF- Insoluble dietary fibre, NDF- Neutral detergent fibre, DMD- Dry matter digestibility, OMD- Organic matter digestibility, M1-M4 List of muesli mixtures containing gluten, M5-M8 List of gluten-free muesli mixtures

The only known treatment for gluten-associated disorders is a life-long wheat exclusion diet, so sample M7 might be recommended for celiacs in terms of a promising source of protein content [12]. Higher fat contents between 6.36-8.78% were recorded in the analysed muesli mixtures if compared with the commercially available samples (5.20%) [13]. Due to these higher fat levels, which are caused by adding oil seeds and nuts, it is strongly recommended to control the products by use of appropriate packaging gas. Muesli mixtures analysed in our study were poor in starch content (from 42.6 to 50.0%).

Focusing on insoluble dietary fibre contents (IDF, complex of lignin and cellulose), the most significant values of up to 1.54% were established in gluten-free M6 and M7 samples (**Table 1**). However, it is generally known that the IDF content in gluten-free food is usually lower than is found in the products containing gluten. Gluten free products are generally richer in fat and sugar and contain less IDF and proteins [14]. Considering neutral-detergent fibre (NDF, complex of lignin, cellulose and hemicellulosis), the highest value of 21.2% was observed in M1 sample. Recently, there has been an increased focus on insoluble parts of dietary fibre because of biological activity of hydroxycin-namic acids, which are esterified to the polysaccharides providing cross-linking between plant cell structures. The health benefits associated with fibre intake are multiple; for instance, reduction of the risk of diseases including type II diabetes, cardiovascular diseases, colorectal cancer etc. [5]. The values of in vitro dry matter (DMD) and organic matter digestibility (OMD) ranged between 80.9-89.8% and 87.8-93.8%, respectively. The highest digestibility values, both DMD and OMD, were monitored in M8 sample. According to Sumczynski et al. [13], high values of DMD and OMD in cereal products positively correlated with low contents of IDF and also with a higher starch content of non-traditional flakes.

## The Evaluation of the Total Phenolic Contents

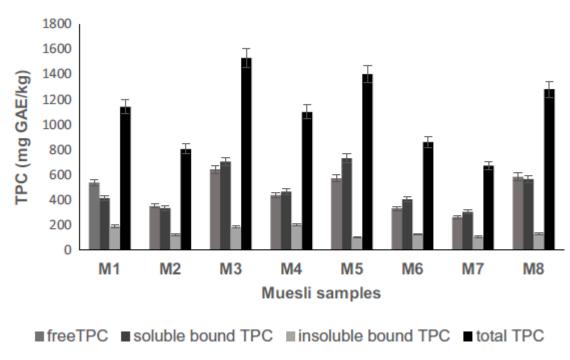
**Figure 2** shows that the values of free, soluble bound and insoluble bound phenolic contents in muesli mixtures reached up to 664, 730 and 200 mg GAE/kg, respectively. The total phenolic content was recorded in a wide range from 671 to 1530 mg GAE/kg with the highest total content established in M3 sample containing oat, rye and kamut flakes, rose, mallow and fruits.

Although data discussing muesli mixtures has been scarce, there are sources confirming that the TPC contents may be influenced by technological processes of raw materials, agricultural machinery or environmental influences, as well as varieties of plants [15].

#### The Evaluation of the Individual Phenolic Acids and Flavonoids Determined by HPLC

Major individual phenolics and their amounts in free, soluble and insoluble bound extracts are depicted in **Tables S2-S4** and chromatograms are shown in **Fig. S1-S3**. A wide array of raw materials applied in the mixtures limits the possibility to compare the results. Therefore, the evaluation of the results obtained in this study focused on phenolics established in different raw materials. Since non-traditional cereals have higher polyphenol contents in free, soluble and insoluble bound phenolic fractions, it is possible to assume that the muesli mixtures should have comparable parameters [16].

Free phenolics determined in high concentrations included quercetin (145 mg/kg), epigallocatechin (98.6 mg/kg), sinapic (49.4 mg/kg) and protocatechuic (27.0 mg/kg) acids.



**Fig. 2** Results of the total phenolic contents (TPC) in individual fractions (free, soluble and insoluble bound). GAE-Gallic acid equivalent, M1-M4 Muesli mixtures containing gluten, M5-M6 Gluten-free muesli mixtures. M1-M4 List of muesli mixtures containing gluten, M5-M8 List of gluten-free muesli mixtures

Both quercetin and epigallocatechin have been identified as one of the most abundant flavonoids in gluten-free cereals [17], similar to edible flowers [18]. High concentrations of a free form of quercetin are typical for Rosa rugosa Thunb. When edible flowers are potential sources of phe-nolics [19] such as were included in our recipes in muesli mixtures. Interestingly, higher values of gallic, caffeic and ^-coumaric acids in free phenolic fractions were recorded in all samples. Concerning the individual total free phenolic contents, the highest value was measured in M8 sample containing rice and quinoa flakes, rose, blue cornflower, blueberries and barberry.

The soluble bound phenolics are shown in **Table S3**. The most abundant phenolics included epigallocatechin (up to 154 mg/kg), chlorogenic acid (up to 83.8 mg/kg) and rutin (up to 73.2 mg/kg). There are many studies declaring the occurrence of these polyphenols in many different sorts of cereals [**20**]; however, data considering the values of soluble bound phenolic forms in fruits and edible flowers has been scarce. Chlorogenic acid has been shown to exert health benefits in the management of obesity, cardiovascular diseases and type 2 diabetes mellitus [**21**].

Quercetin (up to 28.6 mg/kg), protocatechuic acid (up to 14.8 mg/kg) and epigallocatechin (up to 18.4 mg/kg) comprised the major phenolics in insoluble bound fractions (Table S4). It is evident that insoluble bound phenolics were the least represented phenolics compared with the other fractions. The most recurrent bound phenolic acids of edible flowers included protocatechuic, gallic, sinapic, ferulic and *p*-coumaric acids [22]. Considering cereals, black and red rice grains are a valuable source of bound forms of proto-catechuic acid [16, 23]. Since high proportion of phenolic acids are conjugated and bound to dietary fibre, the majority of these compounds reach the colon, and their bioavailability requires the activity of enzymes in intestine and microbiota. After deconjugation, the released phenolic acids can be absorbed across the gastrointestinal barrier and enter the peripheral blood circulation [20, 21].

# The Evaluation of the Individual Anthocyanins Determined by HPLC

It is difficult to compare the anthocyanins and anthocya-nidins values with other already published results due to the variability of the applied methods as well as the composition of muesli mixtures. The individual antho-cyanins and anthocyanidins are presented in Table 2, chromatogram is displayed as Fig. S4. The highest content of cyanidin-3-glucoside of 116.0 mg/kg was determined in M8 sample containing red and black rice flakes, rose, blue cornflower, blueberries and barberry followed by the value of 77.9 mg/kg in M1 muesli sample containing hibiscus, raspberries, barberry and blueberries. Apart from these samples, cyanidin-3-glucoside has been detected as the major anthocyanin also in M2, M4 and M6 samples. Delphinidin-3-glucoside, as the representative of the blue colour, was identified to be the second most frequent anthocyanin constituent in M3 and M8 samples (76.9 and 62.3 mg/kg, respectively). Pelargo-nidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-rutinoside and delphinidin-3-glucoside, representing primary anthocyanins, have been identified in fruits [24]. Furthermore, the individual anthocyanins including cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside, delphinidin, were detected in coloured cereals and edible flowers. Based on already published studies, it may be assumed that the common main sources of anthocyanins in muesli are fruits (e.g., blueberries, raspberries, black currant) and edible flowers. The total and individual anthocyanins contents are influenced by genotype and ripeness of raw materials [19, 25-27].

Table 2 Results of the individual anthocyanin and anthocyanidin contents in non-traditional muesli

Results are presented on dry weight basis as means  $\pm$  SD (n = 5). Means within a line with at least one identical small superscript (in case of all samples) do not differ significantly (P > 0.05), means within a line with at least one identical capitalized superscript (in case of gluten-free and gluten containing mixtures) do not differ significantly (P > 0.05), according to Tukey's test. M1-M4 List of muesli mixtures containing gluten, M5-M8 List of gluten-free muesli mixtures. D-3-glu- delphinidin-3-glucoside, C-3-glu- cyanidin-3-glucoside, C-3-rut- cyanidin-3-rutinoside, P-3-glu- pelargonidin-3-glucoside, Pe-3-glu- peonidin-3-glucoside. ND- not detected, values of LOQs: D-3-glu, P-3-glu, cyanidin, pelargonidin and malvidin 0.01 mg/kg; C-3-rut, Pe-3-glu, peonidin 0.02 mg/kg

Table 2 Results of the individual anthocyanin and anthocyanidin contents in non-traditional muesli

Values (mg/kg)	M1	M2	M3	M4	M5	M6	M7	M8
Anthocyanins								
D-3-glu	$62.0 \pm 1.6^{aA}$	$3.33 \pm 0.14^{b,B}$	76.9 ±0.1°C	ND	71.0±0.6 <sup>d,A</sup>	ND	ND	$62.3 \pm 0.7^{e,B}$
C-3-glu	77.9 ± 1.5 <sup>a,A</sup>	$6.30 \pm 0.10^{b,B}$	72.0 ±0.6°,C	$40.7 \pm 0.6^{d,D}$	53.6±0.4e,A	$33.3 \pm 1.0^{f,B}$	$28.9 \pm 0.5  ^{g,C}$	116±1 <sup>h,D</sup>
C-3-rut	18.6±0.2ªA	ND	$13.2 \pm 1.0^{\text{b.B}}$	ND	16.4±0.3c,A	$1.40 \pm 0.10^{d,B}$	ND	$37.2 \pm 0.6^{e.C}$
P-3-glu	$44.8 \pm 1.3^{a,A}$	ND	$27.7 \pm 1.0^{\text{b.B}}$	ND	$68.7 \pm 1.0^{c.A}$	ND	$44.2 \pm 0.2^{d,B}$	$67.7 \pm 1.0^{e,C}$
Pe-3-glu	21.5 ±0.5 <sup>a,A</sup>	$0.11 \pm 0.01^{b,B}$	9.85 ±0.20°,C	$0.35 \pm 0.05^{d,D}$	12.5 ±0.1 <sup>e,A</sup>	$9.53 \pm 0.26^{f,B}$	ND	13.3 ± 0.1 g,C
Anthocyanidins								
Delphinidin	8.62 ±0.20 <sup>a,A</sup>	$4.31 \pm 0.02^{b,B}$	5.55 ±0.03°,C	$1.43 \pm 0.01^{d,D}$	$6.02 \pm 0.04^{e,A}$	$0.38 \pm 0.02 f^{n,B}$	10.2 ± 0.01 g.C	$6.13 \pm 0.20$ h,D
Cyanidin	5.68 ±0.13 <sup>a,A</sup>	ND	$4.32 \pm 0.16^{b,B}$	0.02 ± 0.01°,C	2.83 ±0.01 <sup>d,A</sup>	ND	ND	ND
Pelargonidin	ND	ND	ND	$0.46 \pm 0.06^{a,A}$	$0.8 \pm 0.03^{b,A}$	$0.04 \pm 0.01^{c.B}$	$0.13 \pm 0.01^{d.C}$	$0.03 \pm 0.01^{e.B}$
Peonidin	ND	0.04±0.01 <sup>a,A</sup>	ND	ND	ND	$0.06 \pm 0.01^{b,A}$	$0.03 \pm 0.01^{c,B}$	≤0.01 <sup>d,C</sup>
Malvidin	0.41 ±0.01 <sup>a,A</sup>	$0.06\pm0.01^{b,B}$	$0.38 \pm 0.02^{c,C}$	$0.88 \pm 0.01^{d,D}$	$0.57 \pm 0.07^{e,A}$	ND	$0.11 \pm 0.01^{f,B}$	$0.66 \pm 0.04$ g,C
Total anthocyanins and anthocya- nidins	240 ± 2 <sup>a,A</sup>	14±1 <sup>b,B</sup>	210 ± 2 <sup>c,C</sup>	44±2 <sup>d,D</sup>	232±3 <sup>e,A</sup>	45 ± 1 <sup>d,B</sup>	84 ± 2 <sup>f,C</sup>	303±g,D

#### Conclusion

Non-traditional flakes and edible flowers have gained the attention and are incorporated into muesli mixtures in order to enhance the nutritional value and attraction of muesli products. This study has reported that muesli mixtures with edible flowers are characterized by high proportions of fibre contents, as well as good digestibility values. Nevertheless, since these raw materials contain higher amounts of lipids, the possibility of oxidative changes during the storage should be considered. The most significant values of the individual phenolic contents were evaluated in free and soluble bound fractions while the lowest concentrations were recorded in bound fractions. Furthermore, this study has provided the analysis of the individual anthocyanins using HPLC. The main antho-cyanins included cyanidin-3-glucoside and delphinidin-3-glucoside. Quercetin and epigallocatechin were the most abundant flavonoids and sinapic and protocatechuic acids represented phenolic acids the most. These findings have proved that non-traditional muesli mixtures might be a valuable source of nutrients and bioactive substances.

They can be recommended for the production in the food industry as unconventional cereal mixtures.

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