Pol. J. Food Nutr. Sci., 2021, Vol. 71, No. 1, pp. 29–37
On-line ISSN: 2083-6007
Print ISSN: 1230-0322
DOI: 10.31883/pjfns/131269
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

Goji Berry and Whey Protein Concentrate Enriched Rice Extrudates – Physical Properties and Accessibility of Bioactives

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Key words: extrudates, goji berries, whey protein concentrate, HPLC, rutin, 2-*O*-β-D-glucopyranosyl-L-ascorbic acid

Extrudates are gaining popularity as convenient ready-to-eat products such as snacks or breakfast cereals. The nutritional limitation of extruded products is their low content of proteins, fibres, and phytochemicals. The challenge lies in increasing the nutritional value of extruded products while maintaining the quality of expansion. Goji berries are rich in bioactive compounds, such as polysaccharides, phenolic compounds, carotenoids, and an analogue of vitamin C. In the present study, rice flour-based extruded products were enriched with goji berries and whey protein concentrate. The varying addition of goji berries and whey protein concentrate affected expansion ratio, colour, and texture parameters of extrudates. The content and bioaccessibility of goji bioactives, *i.e.* 2-*O*-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA) and the dominant phenolic compound – rutin, were evaluated for two extrudates with the highest addition of goji and whey protein concentrate. The extrusion process significantly reduced the content of 2-β-gAA both in formulations with and without whey protein concentrate by approximately 15%. The bioaccessibility of 2-β-gAA was negatively affected by the extrusion process, but not that of rutin. The addition of whey protein concentrate at a level of 7% had no significant effect on the bioaccessibility of neither 2-β-gAA nor rutin.

INTRODUCTION

Ready-to-eat products obtained by the extrusion process are gaining popularity as snacks or alternatives to breakfast cereals. They are eagerly consumed due to convenience and appealing texture characterised by crispiness and crunchiness [Brennan *et al*., 2013]. Extruded products might also respond to the increasing demand for gluten-free products [Alonso dos Santos *et al.*, 2019]. Efforts have been made to enhance their nutritional value by enriching with fibres and phytochemicals from fruits and vegetables [Brennan *et al.,* 2011; Obradović *et al.,* 2015; Wójtowicz *et al.*, 2018]. Extruded products enable also a decrease in sugar content, which is a pertinent requirement for a healthy nutrition [Faruque *et al.,* 2019]. It should be taken into account that the addition of fruits and vegetables decreases the amount of starch in the blend and might significantly influence the physical characteristics of extrudates, such as expansion and colour [Masatcioglu *et al.,* 2013; Obradović *et al.*, 2015; Yu *et al.*, 2017].

Extrusion is a high-temperature-short-time process which has an impact on the content and bioaccessibility of polyphenols [Zeng *et al.,* 2016], proteins, starch, carotenoids, and other compounds [Singh *et al.,* 2007]. The effect of extrusion on the content of phenolic compounds depends on two opposite outcomes: decomposition of heat-labile compounds and disruption of the cell wall followed by a release of covalently bound phenolic compounds [Wang *et al.,* 2014]. Stating about a potential health effect of food components should be preceded by bioaccessibility assessments confirming that after the digestion process the compound of interest is still present and available for absorption. Bioaccessibility is defined as the amount of a compound that is released from the food matrix and is considered to be available for absorption through the gut wall [Fernández-García *et al.,* 2009]. Bioaccessibility depends on processing and interactions with components of the food matrix, such as proteins for example. It may be determined by analysing the *digesta* after an *in vitro* gastro-intestinal digestion using enzymes under controlled conditions such as pH, temperature, ionic strength, and digestion time. Conducting human trials being costly and ethically disputable, simulated *in vitro* digestions have the advantage to be more rapid and less expensive [Minekus *et al.,* 2014].

Gou Qi Zi, *Lycii Fructus*, wolfberry or goji berry are the names given to fruit of *Lycium barbarum*, a plant from the *Solanaceae* family, growing in the temperate and subtropical zones of the world [Levin *et al.,* 2011]. In the traditional Chinese medicine, goji berries are recommended for their capacity to strengthen muscles, protect liver functions, regenerate the vital essence, and improve visual acuity [Huang, 1998]. Due to their potential benefits for human health

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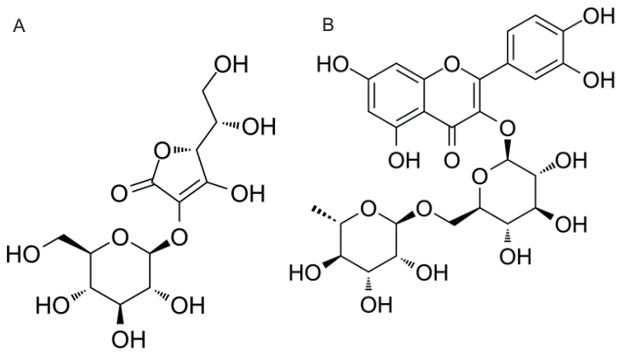

FIGURE 1. Chemical structures of goji bioactives (A) 2-*O*-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA) and (B) rutin.

and thanks to efficient marketing strategies, goji berries are nowadays gaining popularity in western countries [Potterat, 2010]. It has been reported that goji berries contain 2-*O*-β--D-glucopyranosyl-L-ascorbic acid (2-β-gAA), called also an analogue of ascorbic acid [Toyoda-Ono *et al.,* 2004]. The 2-β-gAA molecule consists of an l-ascorbic acid bound to a d-glucose moiety by a β-glycosidic linkage (Figure 1A). The 2-*O*-α-D-glucopyranosyl-L-ascorbic acid, another derivative of ascorbic acid possessing a provitamin C activity and having a similar structure to 2-β-gAA, is widely used in Japan, particularly as a food and cosmetics additive [Yamamoto *et al.,* 1992]. Little is known about the potential provitamin C activity of 2-β-gAA in humans, as no clinical trial has been launched hitherto. Another bioactive compound of goji berry is rutin (Figure 1B), a predominant flavonoid [Potterat, 2010]. Rutin possesses antioxidative, antimicrobial, antifungal, and anti-allergic properties. It can also provide benefits for the treatment of cancer, diabetes, hypertension and hypercholesterolemia [Sharma *et al.,* 2013]. Clinical studies have shown that rutin, in combination with forskolin, a labdane diterpene produced by the Indian coleus plant, could reduce by 15% the intraocular pressure in patients with primary open-angle glaucoma [Vetrugno *et al.,* 2012].

The nutritional limitations of extruded products lie in their low protein contents. Thus, whey protein concentrate (WPC), as a valuable source of proteins and minerals, might be added in order to increase nutritional value of extrudates [Yu *et al.,* 2017]. WPC has a high nutritional quality resulting from its essential amino acid content, especially leucine and lysine, but also valine, threonine, methionine, and phenylalanine [Silva Teba *et al.,* 2017]. Supplementation with whey protein contributes to increased muscle protein synthesis and results in weight loss, satiety, and improved body composition [Fassina *et al.,* 2019]. Increasing the protein content of extruded products while maintaining the quality of expansion is challenging. It was reported that the addition of WPC (up to 7.36 g/100 g) positively influenced the quality of the final extruded product regarding physical properties [Silva Teba *et al.,* 2017].

The aim of this study was to evaluate the effect of goji and WPC addition on the expansion, colour, and texture parameters of extrudates and to determine the effect of extrusion on the content and the bioaccessibility of 2-β-gAA and rutin in goji and WPC enriched extrudates.

MATERIALS AND METHODS

Materials and reagents

Hydrochloric acid, formic acid, and sulfuric acid were obtained from Merck (Zug, Switzerland). Pepsin from porcine stomach mucosa, pancreatin from porcine pancreas, bile salts, sodium chloride, 2,6-di-*tert*-butyl-4-methylphenol (BHT), ascorbic acid, rutin, and acetone were acquired from Sigma-Aldrich (Buchs, Switzerland). Acetonitrile was purchased from Macron Fine Chemicals (Center Valley, PA, USA). Ethanol absolute and sodium hydroxide pellets were obtained from Cochimy (Martigny, Switzerland). All reagents used were of analytical grade or higher. Dry goji berries were purchased from Optymis (Morges, Switzerland). Rice flour was acquired from La Riseria Taverne SA (Taverne, Switzerland), salt from Saline de Bex (Bex, Switzerland), and LEDOR MO 80T WPC from Hochdorf (Hochdorf, Switzerland). Deionised water was obtained using a Milli-Q purification system (Millipore AG, Zug, Switzerland).

Extrusion process

The general flowchart of extrudate production process was depicted in Figure 2. Goji berries were ground in a Cut-o--mat H10 (Kneubühler Gastro Ltd, Luzern, Switzerland) for 10 s and passed through a sieve with a mesh aperture diameter of 2 mm. The powder with a particle diameter less than or equal to 2.0 mm (fine fraction) was stored in a metal bucket. The coarse particles were recovered, and the grinding and the sieving were repeated until the amount of mix was too low to be ground. The coarse particles were thrown away and the fine fractions were combined and stored in the same metal bucket at room temperature without humidity. Batches of 5 kg of raw mixture were prepared. The mixture for the production of rice extrudates (RE) were composed of 99.5% of rice flour and 0.5% of salt. In the mixture for the production of goji enriched extrudates, 3, 7, 10, 13, 17, and 20% of rice flour was replaced by goji powder, whereas for the production of goji and WPC enriched extrudates 2, 4 and 7% of WPC was added. All preparations were mixed in a powder mixer (Prodima, Saint-Sulpice, Switzerland) for 30 s before extrusion.

The extrusion conditions were based on the work of Kosińska-Cagnazzo *et al*. [2017]. Briefly, a K-Tron powder feeder (Coperion K-Tron, Niederlenz, Switzerland) was coupled with an Evolum 25 twin screw extruder (Clextral, Firminy, France). The screw configuration allowed applying a high shear to the material. The temperatures in the five first barrels from the feeder to the die were as follows: 20, 40, 60, 80, and 100°C. The last five barrels as well as the die, which was round and had a 2 mm diameter, were heated at 140°C. The dry mixture was fed at 13 kg/h and water was pumped at 1.4 L/h (DKM piston metering pump, Firminy, France). The speed of the extruder screws was set at 400 rpm and allowed to have a constant feed. A pelletizer EX21 (Clextral, Firminy, France) set at 2,000 rpm was placed in the front of the die and cut the extrudates at the exit. Die pressure and specific mechanical energy (SME) was constantly measured during the extrusion process. To prevent agglutination of the hot, humid and therefore sticky extrudates, a ventilation of 2000 L of air/min was applied at ambient temperature.

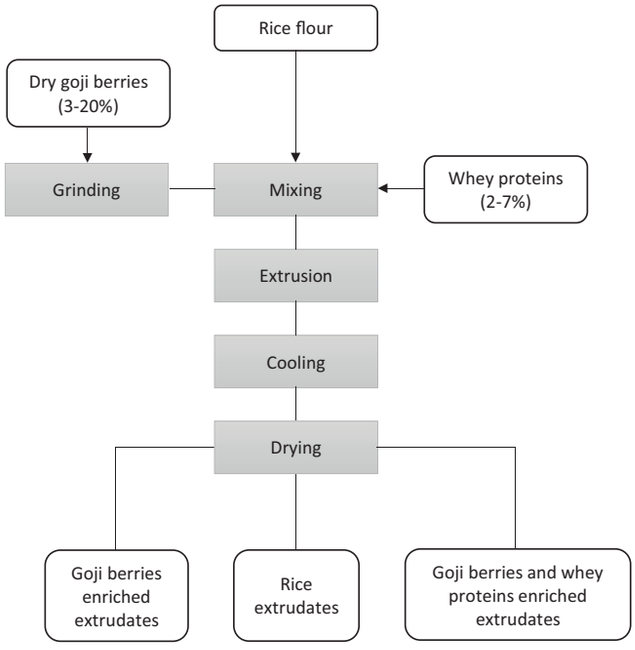

FIGURE 2. Flowchart of the extrusion process.

The cut extrudates were collected, cooled down, and dried in an Euromat B4 IS600 oven (Wiesheu, Grossbottwar, Germany) at 120°C for 10 min to obtain a final moisture level lower than 5%. Once the extrudates reached the room temperature, they were placed in plastic bags, sealed, and stored at 4°C until analysis. The water content of goji berries, pre-extrusion mixtures, and extrudates was evaluated using an HG53 Halogen moisture analyser (Mettler Toledo, Greifensee, Switzerland).

Physical characterization of extrudates

Expansion ratio

The diameters of 15 pieces of each extruded product were measured with a calliper (Mitutoyo, Urdorf, Switzerland). Diametric expansion ratio was calculated as a cross-sectional diameter of an extrudate divided by the diameter of the die opening and multiplied by 100.

Colour parameters CIELAB

Finely ground extrudates (5 g) were analysed in triplicate using a Konica Minolta CM-5 (Chiyoda, Tokio, Japan) colorimeter with standard illuminant D65 and 10° observer. The values of lightness (L*), greenness/redness (a*), and blueness/yellowness (b*) were recorded. Analysis was performed in 5 replicates.

Hardness analysis

Texture analyser TA-XT (Stable Micro Systems, Godalming, United Kingdom) was employed. An Ottawa cell of 9×7.1×7.1 cm was filled with extrudates and hardness analysis was performed by compression by 25% at a speed of 5 m/s. Analysis was performed in five replicates and the results were expressed in N.

Bulk density

An Ottawa cell was filled up with extrudates and the weight was evaluated in ten replicates. The results were expressed as kg/m^3.

In vitro digestion

A two-stage *in vitro* digestion model based on the procedure described by Xie *et al*. [2013] was used with some modifications. The gastric phase was initiated by weighing 4 g of ground samples (dry goji berries, pre-extrusion mixtures or extrudates) into a 50 mL tube. Then, 15 mL of 0.05 mol/L HCl solution (pH 1.3) was added to the samples (20 mL for the extrudates). A pepsin solution (8 mL; 3.5 g of pepsin from porcine stomach mucosa in 500 mL of 0.1 mol/L HCl) was added, the mixture was purged with nitrogen and placed in a Multitron PRO incubator (Blanc Labo, Lonay, Switzerland) at 37°C for 1 h with continuous shaking. After the gastric digestion, the pH was adjusted to 6.5 using an NaOH solution (1 mol/L). After addition of 8 mL of a pancreatin and bile salts solution (3.5 g and 3.7 g respectively in 500 mL of 0.1 mol/L $NaHCO_3$), the mixture was purged with nitrogen and placed at 37°C for 2 h with continuous shaking. The solution was centrifuged at 4,000 × *g* for 15 min using an Eppendorf Centrifuge 5810 (Hamburg, Germany) and the supernatant was recovered in a volumetric flask. The solution was adjusted to the volume of 50 mL with 0.9% NaCl solution and stored at 4°C until analysis. During the whole digestion process, the solutions were protected from light.

Chemical analyses

Extraction and analysis of 2-O-β-D-glucopyranosyl-L-ascorbic acid

The procedure of extraction was based on a previously published method [Kosińska-Cagnazzo *et al.,* 2017]. The samples of 1 g of dry goji berries, pre-extrusion mixture, and extrudates, respectively, were extracted with 10 mL of water in an ultrasonic bath for 10 min. Following centrifugation at 4,000 × *g* for 5 min, the supernatant was recovered in a 25 mL volumetric flask. The extractions from the residue were performed twice more with 5 mL of water and the supernatants were combined in the 25 mL graduated flask. Oxalic acid solution (1 mL, 4% *w/v* in water) was added to stabilize the solution and the volume was adjusted to 25 mL with water. The extractions were performed in quadruplicates for each sample. The extracts were filtered through a 0.20 μm PTFE syringe filter (Chromafil, Macheret-Nagel, Düren, Germany) in standard vials and stored at 4°C until analysis.

For digested samples, 2 mL of digesta was sampled and mixed with 2 mL of ethanol absolute in a 15 mL tube. The solution was then centrifuged at 4,000 × *g* for 3 min and the supernatant was collected and filtered through a 0.20 μm PTFE syringe filter (Chromafil, Macheret-Nagel) into a standard vial and stored at 4°C until analysis. The extractions were carried out in triplicates.

A volume of 5 μL of extracted and digested samples was injected onto an amino column (Aminex HPX-87H Ion exclusion, 300 mm × 7.8 mm i.d., particle size 5 μm, Bio-Rad, Hercules, CA, USA) coupled to a precolumn. The diode array detector (DAD) was set at 210 and 254 nm for the

detection of the analogue. The mobile phase was a 5 mmol/L sulfuric acid solution delivered in isocratic mode. The flow rate of 0.5 mL/min was applied and separation was carried out at 35°C. The quantification of 2-β-gAA was made using a calibration curve for ascorbic acid and a conversion factor reported by Tai & Gohda [2007]. The results were expressed as μg/g of goji berries dry matter (DM).

Extraction and analysis of rutin

The procedure was based on the method of Kosińska-Cagnazzo *et al.* [2017] with some modifications. Briefly, 600 mg of dry goji berries or 1,200 mg of pre-extrusion mixtures or extrudates was extracted with 5 mL of 70% (*v/v*) ethanol in an ultrasonic bath (VWR, Dietikon, Switzerland) at a 45 kHz working frequency for 10 min. Following centrifugation at 4,000 × *g* for 5 min, the supernatant was recovered in a 20 mL graduated flask. The extractions from the residue were performed twice more with 5 mL of 70% (*v/v*) ethanol and the supernatants were combined in the 20 mL graduated flask. The volume was adjusted to 20 mL using 70% ethanol. The extractions were performed in quadruplicates for each sample. The extracts were then filtered through an Exapure 0.45 μm nylon syringe filter (Alys Technologies, Bussigny, Switzerland) into vials and stored at 4°C until analysis. Digesta were extracted as described in the "*In vitro* digestion" section.

An Agilent 1220 infinity series liquid chromatograph (Agilent Technologies, CA, USA) coupled to an autosampler, a binary pump, and a G4294B UV-DAD detector (Agilent Technologies 110 Series) was used to carry out the HPLC analyses. A volume of 1 μL of extracts and 2 μL of digesta was injected onto a Kinetex C18 column (2.6 μm, 50 mm × 2.1 mm; Phenomenex, Torrance, CA, USA) heated at 40°C. The DAD was set at 320 and 340 nm for the detection of rutin. The mobile phase A was made of 1% aqueous formic acid and the mobile phase B of 1% (*w/v*) formic acid in acetonitrile. The elution gradient was as follows: 0 min: 100% A, 2 min: 100% A, 25 min: 90% A, 26 min: 90% A, 30 min: 40% A, 35 min: 40% A, 35.1 min: 100% A. The flow rate of 0.3 mL/min was applied. The identification was performed by comparison of UV-Vis spectra and retention time with those of standard compound of rutin. Quantification was done by external calibration. The results were expressed as μg/g DM of goji berries.

Statistical analysis

The extrusion process was performed in duplicate on two different days. The results are expressed as mean ± standard deviation. One-way ANOVA followed by a post hoc Tukey test was performed to determine if the differences between the results were statistically significant. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Extrusion process characteristics and physical properties of extrudates

The extrusion process was controlled by permanent measurement of die pressure and specific mechanical energy (SME). The collected dataset was compiled in Table 1. The maintaining of the process parameters at the desired values allows obtaining the final product with high overall quality [Moscicki, 2011]. The increasing addition of goji berries led to diminished values of die pressure and SME compared to plain rice extrudates (RE). It was reported that the addition of fruits can act as a lubricant and reduce friction in the extruder and shear forces, and thereby diminish the extrusion pressure [Moscicki, 2011]. WPC addition lowered die pressure even further, with low impact on SME values compared to goji extrudates (Table 1). A recent study evaluated the impact of WPC addition (0–40%) on the extrusion process of corn starch and reported that SME diminished significantly up to a WPC level of 20% [Yu *et al.,* 2017]. The authors attributed this observation to the lower starch content and the lower degree of gelatinization due to water competition between the WPC and corn starch in the blend.

TABLE 1. Extrusion process parameters.

	Goji berries (%)	WPC (%)	Pressure (bar)	SME (Wh/kg)
Rice extrudates	0	0	293.2±5.8^{a}	85.2±3.5^{a}
Rice extrudates with goji	3	0	280.5±4.4^{b}	85.2±3.5^{a}
	7	0	271.7±6.6^{b}	86.4±3.2^{a}
	10	0	243.1±4.3^{c}	78.2±1.7^{c}
	13	0	248.9±3.8^{c}	82.6±1.0^{b}
	17	0	233.6±4.0^{d}	70.2±1.7^{d}
	20	0	212.6±2.4^{e}	64.8±1.4^{e}
Rice extrudates with goji and WPC	20	2	200.2±3.2^{f}	67.6±1.2^{d}
	20	4	195.9±4.6^{fg}	67.7±4.0^{de}
	20	7	192.8±3.4^{g}	68.6±0.9^{d}

SME – specific mechanical energy, WPC – whey protein concentrate. The results with different letters within column are significantly different at $p \leq 0.05$.

The pelletizer employed in the presented study allowed obtaining ready-to-eat extruded products of round shape. A good expansion ratio of about 450% was noted for RE (Figure 3). The addition of 3 and 7% of goji berries to the extrudate blend caused an increase in the expansion ratio, whereas higher addition levels caused the expansion ratio to decrease significantly. Similarly, an initial increase in the expansion ratio with the addition of mango peel powder was observed by Mohamad Mazlan *et al.* [2019]. The linear expansion started to decrease when the addition of mango peel powder exceeded 8.34%. In the present study, 4 and 7% addition of WPC to the blends containing 20% of goji fruits increased the expansion ratio of the extrudates (Figure 3). Some literature data shows an increase in the expansion ratio with the addition of whey proteins up to 10% [Yu *et al.,* 2017].

The colour parameters of extruded products were significantly affected by goji and WPC addition (Table 2). RE showed a high L* value (85.0), which corresponds to lightness, whereas the intensiveness of red colour indicated by a* value was very low (0.96). The addition of goji berries decreased significantly the lightness of the extruded products and increased their

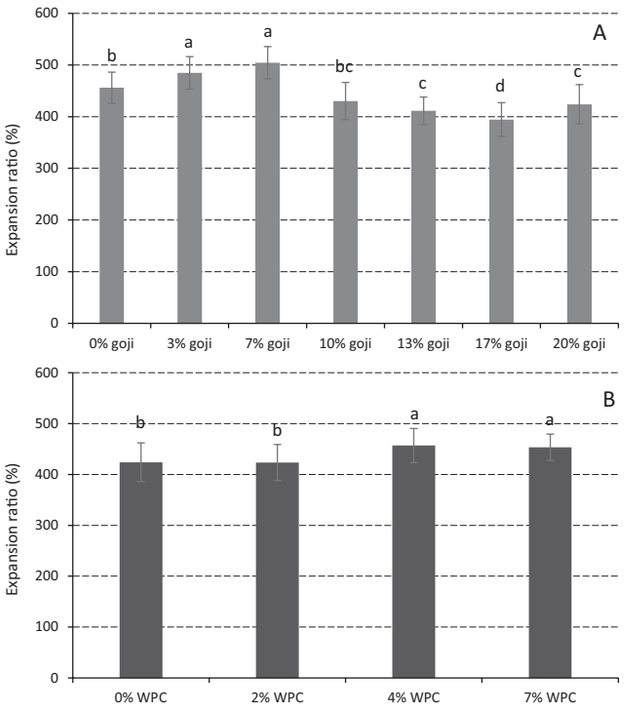

FIGURE 3. Expansion of extrudates with different percentage of goji berries and no whey protein concentrate (WPC) added (A) and with different percentage of WPC and 20% of goji berries (B) The results with different letters are significantly different at p≤0.05.

yellowness (b*) and redness (a*). This result is not surprising regarding the high content of orange-red carotenoids like zeaxanthin dipalmitate in goji berries [Potterat, 2010]. The addition of WPC led to a further decrease in lightness as well as a decrease in values of b* and a* parameters. Darker colour of the extrudates with WPC added might result from the reactions between reducing sugars and amino acids, including Maillard reaction and caramelization [Yu *et al.,* 2017].

Bulk density and hardness were determined for the rice extrudates (RE) and for the extrudates with the maximal addition of goji berries and WPC, *i.e.* rice extrudates with 20% of goji berries added (RGE) and rice extrudates with 20% of goji berries and 7% of WPC added (RGWE). The results were compiled in Table 3. The addition of goji berries as well as the addition of WPC increased significantly bulk density of rice extrudates. Masatcioglu *et al*. [2013] reported no change in bulk density of corn extrudates after adding 8% of ginseng powder and green tea, whereas Wójtowicz *et al*. [2018] found doubled values of bulk density after 10 to 20% addition of tomato powder to corn extrudates. The addition of 20% of goji berries increased the hardness of rice extrudates in comparison to RE. However, when goji berries were added together with whey proteins (RGWE) the increase in hardness was not significant. The addition of mango peel powder at 25% was observed to increase the hardness of extrudates up to 380 N [Mohamad Mazlan *et al*., 2019]. In the present study, a decrease in the expansion ratio was observed together with an increase in hardness in RGE in comparison to RE. A similar effect was observed by Wójtowicz *et al*. [2018] when corn extrudates were enriched with powdered tomato.

TABLE 2. Colour parameters of extrudates.

	Goji berries (%)	WPC (%)	*L**	*a**	*b**
Rice extrudates	0	0	85.0±1.0^{a}	0.96±0.1^{g}	16.7±0.8^{d}
Rice extrudates with goji	3	0	68.1±1.1^{b}	12.5±0.4^{f}	44.7±1.0^{b}
	7	0	61.6±1.6^{c}	14.7±1.1^{e}	44.6±1.0^{b}
	10	0	59.4±0.6^{d}	17.2±0.5^{d}	48.2±1.5^{a}
	13	0	58.2±0.8^{d}	17.6±0.3^{d}	47.2±0.6^{a}
	17	0	56.8±1.4^{e}	19.1±0.8^{bc}	47.3±1.0^{a}
	20	0	54.9±0.5^{f}	21.6±0.3^{a}	47.9±1.0^{a}
Rice extrudates with goji and WPC	20	2	52.2±1.0^{g}	19.4±0.2^{b}	45.2±1.1^{b}
	20	4	52.4±1.0^{g}	19.6±0.8^{b}	45.5±1.7^{b}
	20	7	49.8±1.0^{h}	18.8±0.2^{c}	41.8±1.4^{c}

WPC – whey protein concentrate. The results with different letters within column are significantly different at $p \leq 0.05$.

Content of 2-*O*-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA)

Goji berries, pre-extrusion mixture, RGE, and RGWE were analysed for the content and bioaccessibility of bioactives.

The content of 2-β-gAA in goji berries, pre-extrusion mixture, and extruded products was evaluated after aqueous extraction. Dry goji berries contained about 2,920 μg/g of 2-β-gAA. As expected, the pre-extrusion mixture of RE and RE itself contained no 2-β-gAA. The content of 2-β-gAA in the pre-extrusion mixture powder, amounted to 594 and 549 μg/g DM for RGE and RGWE, respectively (Figure 4A). The addition of 7% WPC, replacing rice flour, did not affect the content of 2-β-gAA in the pre-extrusion mixtures ($p>0.05$). On the other hand, the extrusion process significantly reduced the content of 2-β-gAA both in RGE and RGWE by approximately 15%. Similarly, as in the case of pre-extrusion mixtures, the content of 2-β-gAA did not differ significantly ($p>0.05$) between RGE and RGWE.

Compared to ascorbic acid, which is sensitive to heat and light, 2-β-gAA showed quite good stability during extrusion, as revealed previously [Kosińska-Cagnazzo *et al.,* 2017]. The die temperature, moisture content, speed and geometry of extruder screws influence significantly the content of ascorbic acid in extruded products with losses up to 76% [Obradović *et al.,* 2015]. The instability of ascorbic acid comes from the hydroxyl groups of the 2,3-enediol, which are easily oxidized, leading to dehydroascorbic acid. Numerous ascorbic acid derivatives possessing substituents at these positions, such as phosphate, sulphate, galactose or glucose [Yamamoto *et al.,* 1990], turn out to be more stable than ascorbic acid, which can explain why 2-β-gAA was more stable during extrusion.

Bioaccessibility of 2-*O*-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA)

After *in vitro* simulated gastrointestinal digestion, 2,460 μg/g of 2-β-gAA was detected in dry goji berries digesta, which was not significantly different from the content of

TABLE 3. Bulk density and hardness of extrudates.

Extrudates	Bulk density (g/cm^3)	Hardness (N)
RE	35.5±3.24^c	167±24^b
RGE	50.3±4.35^a	215±28^a
RGWE	40.3±5.44^b	181±40^{ab}

RE – rice extrudates, RGE – rice extrudates enriched with goji berries (20%), RGWE – rice extrudates enriched with goji berries (20%) and whey protein concentrate (7%). The results with different letters within column are significantly different at $p \leq 0.05$.

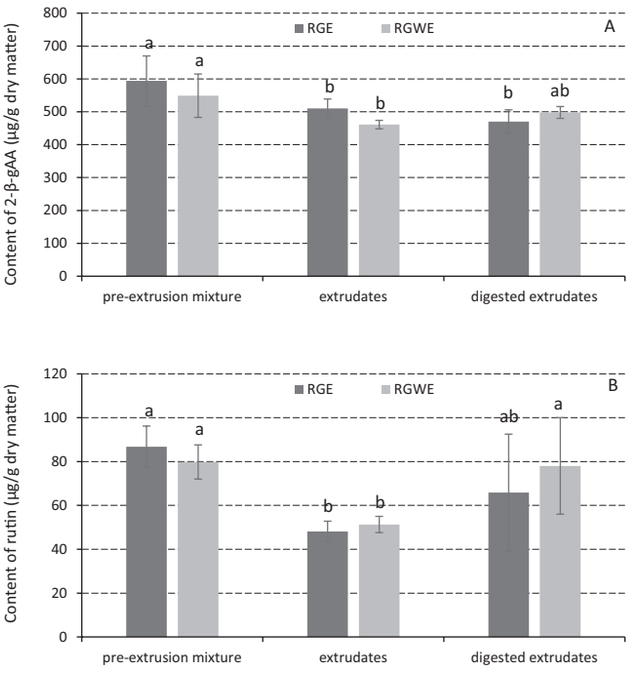

FIGURE 4. Content of 2-*O*-β-D-glucopyranosyl-L-ascorbic acid (2-β-gAA) (A) and rutin (B) in extrudates as influenced by extrusion and digestion process RGE rice extrudates enriched with goji berries (20%) RGWE rice extrudates enriched with goji berries (20%) and whey protein concentrate (7%) The results with different letters are significantly different at p≤0.05.

2-β-gAA in goji berries before digestion ($p>0.05$). The addition of WPC at the level of 7% did not significantly affect the bioaccessibility of 2-β-gAA from extrudates (Figure 4A), which amounted to 470 and 498 μg/g DM for RGE and RGWE, respectively. There was no statistically significant difference in the content of 2-β-gAA before and after *in vitro* digestion for both RGE and RGWE. It might be due to the subcellular distribution of 2-β-gAA in fruits. It is assumed that ascorbic acid is synthesised by the plants in the cytosol through three different pathways and transported into chloroplasts by facilitated diffusion [Rautenkranz *et al.,* 1994]. If the synthesis of 2-β-gAA in goji berries occurs also in the cytosol, this means that the substance can easily diffuse from the cytosol to the extraction solvent. Thus, in general, 2-β-gAA is not bound to other compounds. Therefore, enzymes present during the *in vitro* digestion do not liberate more 2-β-gAA from the food matrix and, at the same time, 2-β-gAA is stable under digestion conditions and does not undergo degradation.

Concerning the nutritional values, ascorbic acid derivatives might be transformed to active vitamin C in the human body, due to the activity of such enzymes as phosphatase, sulfatase, α-glucosidase, and β-galactosidase [Nakamura *et al.,* 2009]. Three native β-glucosidases are present in the human body. Glucocerebrosidase and lactase phlorizin hydrolase are a part of the brush border enzymes, whereas the last β-glucosidase is a broad-specificity cytosolic enzyme present in the liver, kidney, and small intestine. The cytosolic β-glucosidase has been shown to cleave β-linkages in some isoflavonoids [Day *et al.,* 1998]. Nonetheless no studies have been performed on the ability of those enzymes to hydrolyse 2-β-gAA into ascorbic acid and glucose in human. Toyoda-Ono *et al.* [2005] reported an increased level of ascorbic acid and of intact 2-β-gAA in the portal vein blood of rats, after oral administration of the analogue. According to their findings, 2-β-gAA to some extent maintains the level of ascorbic acid in the rat tissues and hence acts as a provitamin C. They even proposed that the 2-β-gAA might serve as a stable ascorbic acid substitute for clinical applications. It remains to be evaluated whether 2-β-gAA might have provitamin C activity and, in consequence, whether goji berries and their extrudates are a reliable source of ascorbic acid in humans.

Content of rutin

The content of rutin in dry goji berries amounted to 408 μg/g DM. The addition of WPC at a level of 7% did not influence significantly ($p>0.05$) the extractability of rutin from pre-extrusion mixtures or extrudates (Figure 4B). The extrusion process decreased significantly the content of rutin in both types of extrudates, from 86.8 to 48.1 μg/g DM and from 79.8 to 51.3 μg/g DM for RGE and RGWE, respectively. The retention of around 60% of rutin from goji berries during the extrusion process corresponds with the existing literature [Kosińska-Cagnazzo *et al.,* 2017; Leyva-Corral *et al.,* 2016]. It is known that the extrusion cooking process might cause a decrease in the content of some compounds, such as polyphenols, which is generally attributed to the high temperature process [Ti *et al.,* 2015]. However, other parameters may influence the level of bioactive compounds in extruded products, such as the shear due to the screw configuration, the screws speed, and the moisture content [Brennan *et al.,* 2011]. For example, the stability of anthocyanins depends on die temperature and moisture content, *i.e.* the higher the die temperature, the lower the content of anthocyanins. The increase of moisture content has the opposite effect; the higher moisture content allows retaining more compounds after the extrusion cooking process [Durge *et al.,* 2013]. Oxidation of flavonoids in foods can occur through exposure to high pH, heat, and oxygen reactive species or when flavonoids come into contact with degradative enzymes, such as polyphenol oxidase, after cell wall degradation. Consequently, quinones are formed and may covalently bind to protein amine and amide groups, which may reduce the content of accessible flavonoids [Bordenave *et al.,* 2014]. Furthermore, during roasting, rutin may be converted into its aglycone, quercetin, which might also occur during the drying at 120°C for 10 min. The effect of roasting of flavonoid glycoside of noni leaves was investigated, and ru-

tin content was observed to decrease after a 20-min thermal treatment in an oven [Deng *et al.,* 2011]. A similar effect was observed for quercetin glycosides of onions during the roasting process, which suggests that flavonoid glycosides present in goji berries might follow the same path [Rohn *et al.,* 2007].

Bioaccessibility of rutin

The amount of rutin released from dry goji berries during *in vitro* simulated gastrointestinal digestion amounted to 227 μg/g and was significantly lower than its content in goji berries. The bioaccessibilty of rutin from RGE and RGWE amounted to 65.9 and 78.0 μg/g DM, respectively (Figure 4B). Interestingly, the amount of rutin released from RGWE during digestion was higher than that extracted from the same sample with a solvent. The addition of WPC at a level of 7% did not influence the bioaccessibility of extrudates.

Thermal processes have an impact on flavonoids structure and may also influence their bioaccessibility and bioavailability [Rohn *et al.,* 2007]. Only 17% of accessible rutin is absorbed by the small intestine, whereas the rest is hydrolysed into quercetin and metabolised by faecal microflora in phenylacetic acids such as 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid, and 3,4-dihydroxyphenylacetic acid [Olthof *et al.,* 2003]. The content of rutin in the digesta of boiled potato accounted for 169% of the initial amount of rutin present. Polyphenol concentration in boiled potatoes issued from the chemical extraction may therefore underestimate the actual amount that can be released during digestion and that are bioaccessible [Miranda *et al.,* 2013]. Also in blended fruit juices the content of chlorogenic and *p*-coumaric acids, as well as naringenin and rutin increased after simulated gastrointestinal digestion [Rodríguez-Roque *et al.,* 2013]. On the other hand, in the same study, the content of ferulic and sinapic acids, hesperidin, quercetin, and (+)-catechin was lower in the digesta than in the juices before digestion.

The subcellular distribution of rutin on the goji shrub may play a role as well. The molecule is synthetised in the endoplasmic reticulum, possibly in a multi-enzyme complex [Falcone Ferreyra *et al.,* 2012]. Contrarily to ascorbic acid, which is synthetised in the cytosol and transported through facilitated diffusion, rutin and other polyphenols would be translocated from endoplasmic reticulum to vacuole or cell wall by an active process [Mintz-Oron *et al.,* 2008], possibly by the action of a multidrug resistance-associated protein transporter [Petrussa *et al.,* 2013], which could make it harder to extract. In food, non-covalent binding may occur between flavonoids and other macronutrients, such as proteins for example, involving Van der Waals forces such as hydrogen bonding as well as ionic and London interactions [Bordenave *et al.,* 2014]. This might reduce the amount of accessible rutin. Despite the above-mentioned potential interactions between proteins and flavonoids, a content of 7% WPC seems to have no significant influence on the extractable amount of rutin from extruded materials. However, according to Bordenave *et al.,* [2014], interactions between milk proteins and flavonoids might allow to have a better stability over storage time, by decreasing the availability of flavonoids for oxidative reactions.

CONCLUSION

The addition of goji berries at up to 7% improved the expansion ratio of the rice extrudates. Colour parameters were highly affected by the addition of goji berries and WPC. Extrusion is a high-temperature-short-time process that decreases significantly the content of 2-β-gAA and rutin coming from goji berry addition. 2-β-gAA was retained at 85% after the extrusion process, which suggests that the compound is more stable to heat, light, and oxygen than ascorbic acid. Bioaccessibility of 2-β-gAA from goji berries, but not that of rutin, was affected by the extrusion process. The addition of WPC at a level of 7% showed no significant effect on the bioaccessibility of neither 2-β-gAA nor rutin. The results suggest that the interactions between WPC and 2-β-gAA or rutin did not impact their bioaccessibility. The release of 2-β-gAA and rutin during *in vitro* simulated digestion was comparable to that of a chemical extraction carried out.

In view of the potential health benefits of the goji compounds, additional studies should be conducted, using the Caco-2 cell culture model, in order to predict their *in vivo* intestinal absorption. Provided that 2-β-gAA and rutin are well absorbed, the extrudates could be a reliable source of these beneficial compounds.

RESEARCH FUNDING

The study was financially supported by the HealthFood – Thematic Research Programme of University of Applied Sciences and Arts Western Switzerland.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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Submitted: 20 October 2020. Revised: 7 December 2020. Accepted: 8 December 2020. Published on-line: 22 January 2021.