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Possibility of Water Saving in Processing of Snack Pellets by the Application of Fresh Lucerne Sprouts: Selected Aspects and Nutritional Characteristics

Maciej Combrzyński[1], Agnieszka Wójtowicz[1], Beata Biernacka[1], Tomasz Oniszczuk[1*], Marcin Mitrus[1], Jakub Soja[1], Renata Różyło[2], Karolina Wojtunik-Kulesza[3], Kamila Kasprzak-Drozd[3], Anna Oniszczuk[3]

[1] Department of Thermal Technology and Food Process Engineering, University of Life Sciences in Lublin, Głęboka 31, 20-612 Lublin, Poland

[2] Department of Food Engineering and Machines, University of Life Sciences in Lublin, Głęboka 28, 20-612 Lublin, Poland

[3] Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland

* Corresponding author's e-mail: tomasz.oniszczuk@up.lublin.pl

ABSTRACT

New types of extruded snack pellets of wheat-corn blend base and fresh lucerne sprouts were developed. The aim of the study was to examine the effect of fresh lucerne sprouts addition on the water consumption, processing efficiency and the specific mechanical energy during production of wheat-maize snack pellets. Additionally, the total phenolic content and antiradical activity, as well as the water absorption and water solubility indices in samples processed under variable processing conditions were tested. The extrusion-cooking of blends consisted of 10, 20 and 30% of lucerne sprouts was carried out using a single screw extruder at screw speeds of 60 and 100 rpm, and at moisture contents of 32, 34 and 36%. Replacement of wheat-corn flour blends by fresh lucerne sprouts at various levels (10, 20 and 30%) enabled to sufficiently reduce technological water which is needed in extrusion-cooking process of snacks pellets. The limitation of water was from 89 to 100% if fresh lucerne sprouts were used, depending on the recipe and dough moisture level tested. Total phenolic content and antioxidant activity increased significantly due to lucerne sprouts addition. Furthermore, higher water absorption and water solubility index were noted if increased initial moisture content was applied during the processing of snack pellets. It can be concluded that fresh lucerne sprouts can be valuable additives, enabling to save the technological water in production process and to obtain nutritionally valuable supplemented wheat-corn-based snack pellets.

Keywords: technological water; lucerne; *Medicago sativa* L.; extrusion; snack pellets; antioxidants; functional food

INTRODUCTION

One of today's greatest challenges is to provide valuable food for the growing number of people on our globe with no negative environmental impact. Due to the climate change, among others, the limited access to fresh water is observed in many countries. Scientists around the world are faced with the enormous problem of ensuring food security and the availability of freshwater resources. Animal husbandry and cultivation of traditional crops involve huge demands on water and energy. One of the solutions to this problem could be hydroponic plant cultivation, which saves up to 95% water compared to traditional methods, as well as the direct use of fresh fruits, vegetables and especially plant sprouts in the agri-food industry to produce nutritionally valuable and environmentally friendly food and feed products [Elmulthum et al., 2023; Stachowski et al., 2023]. Highly efficient agriculture generates huge amounts of biomass every year, which can be used e.g. for methane fermentation. Agricultural biogas plants can utilize surplus biomass for energy purposes [Kalinichenko and Havrysh, 2019]. Shah et al. [2022]. The energy generated in this way can be used in the processing and production of food with specific quality parameters, where production technology is very energy and water consuming. Sustainability policies require control of water and energy flows in the food industry. By using innovative food processing and production technologies, a significant reduction in water and energy demands can be achieved [Lisiecka and Wójtowicz, 2020].

One of the valuable plants with high production efficiency and low cultivation requirements is lucerne. Lucerne is used as a functional food in the form of protein concentrates, extracts or dried leaf powders. For consumption purposes, lucerne seeds undergo various processing techniques (wet heating, microwave treatment, dry heating, soaking or extrusion) in order to reduce the content of anti-nutritional substances and improve the technological properties and bioactive potential [Lai et al., 2020].

Lucerne has been utilized in traditional medicine and phytotherapy. Studies have indicated that lucerne concentrate can be used as a dietary supplement to help humans counter malnutrition, ischemia and numerous diseases. Research indicates that the constituents of lucerne concentrate strengthen human resistance to infections and many diseases, and that the iron concentration improves the hemoglobin content in blood, hence aiding in the control of anemia caused by hemoglobin shortage. In various studies, the saponins and flavonoids in lucerne concentrate have been found to have a positive effect on human health and help strengthen the immune system [Gaweł et al., 2017]. In addition to proteins, lucerne extracts and concentrates contain a variety of vitamins (A, B, C, D, E, K), minerals (Ca, Cu, Fe, Mg, Mn, P, Zn, Si), phytochemical substances (carotene, chlorophyll, coumarins, isoflavones, alkaloids, saponins) and antinutritional components (phytates, L-canavanine, saponins).What is more, this plant is high in dietary fiber and other active components [Shah et al., 2020]. Caunii et al. [2012] discovered that lucerne extract included free hydroxyl groups (hydrogen donors), which conferred the product with substantial antioxidizing activity in the human body. It should be noted that the presence of oxidizing substances in the human body causes cell damage (e.g., DNA mutation) and the development of numerous chronic diseases such as arteriosclerosis, arthritis, diabetes, and cancer. Antioxidants, both synthetic and natural, are given to the diet to prevent the aforementioned conditions [Labhar et al., 2023].

Apart from the seeds or extracts, lucerne sprouts are well-known products with high nutritional value. Previous research has discovered that sprouting of lucerne seeds enhanced the content of vitamins, minerals, and phytoestrogens, boosting their nutritional and health value when compared to fresh plant material. Lucerne sprouts include a wide range of flavonoids, phenolic acids and terpenoids, with calycosin, methyl gallate and epicatechin 3-gallat being the top three elevated. Lucerne sprouts are characterized by high antioxidant activity [Zincă et al., 2013] and are also an excellent source of estrogenic bioactive chemicals [Hong et al., 2011]. Despite the proven nutritional value of lucerne and derived products, they are rarely used as a functional food additive for human consumption [Aloo et al., 2021]. Attempts have been made to add lucerne leaf powder to vegan chocolate; however, lucerne protein isolate has been included within cereal bars [Sahni et al., 2022]. Fresh sprouts are nutritious foods that are low in fat and high in phytochemicals, but they have a short shelf life, necessitating the development of processing procedures that retain their nutritional value [Hong et al., 2011].

To prevent the short shelf-life of sprouts, drying into powder by various methods can be applied, but this generates additional energy consumption. A new way to circumvent this loss could be an incorporation of fresh sprouts into products processed via HTST (high temperature short time) treatment such as through the extrusion-cooking [Shirazi et al., 2020]. This technology allows an intensive treatment with a very short processing time, so it has low energy requirements and moreover a limited destructive effect on the biologically active substances present in the extruded materials. Additionally, this treatment technology may increase the extractability of bioactive compounds. This is very profitable when fruit, vegetable or herbs are added as functional components, because of limited changes in bioavailability of the extruded components, especially when low temperature extrusion is applied as in the processing of snack pellets [Soja et al., 2023]. Expanded puffs can be directly created as a result of low-moisture (12–18%) high-shear extrusion-cooking at high temperature, but also they can be produced as indirectly expanded crisps from snack pellets created through the use of low-temperature semi-moisture (25–36%) extrusion technology [Prabha et al., 2021]. The snack pellets may be then expanded by many ways, the most popular being hot oil frying or low-fat expansion by toasting or microwaving [Kraus et al., 2014; Lisiecka et al., 2021].

As stated by Lisiecka and Wójtowicz [2020], the use of fresh vegetables can significantly reduce the water volume needed in the technological process and the energy needed for the drying process. Thus, the incorporation of fresh lucerne sprouts as an additive to snack pellets processed via extrusion-cooking may have many advantages, both processing and nutritional. What is more, after drying, the pellets have moisture content below 11%, making the products stable and safe for long storage.

The aim of the study was to develop new type of wheat-maize-based snack pellets fortified with fresh lucerne sprouts addition and to evaluate the effects of lucerne sprouts level as well as processing conditions on water consumption, processing aspects and selected physiochemical characteristics of the obtained snack pellets. There are no current studies on the use of fresh lucerne sprouts as an additive to extruded snack pellets; thus, the present study is a first.

MATERIALS AND METHODS

Raw materials and snack pellets processing

The main components of snack pellets were wheat flour type 450 and corn flour (50:50), sugar (2%) and salt (1%), purchased at the local market. Basic composition of the used wheat flour was: moisture 11.40%, protein 12.26%, fat 0.98%, ash 0.45%, fiber 1.30%. That of corn flour was: moisture 9.88%, protein 5.13%, fat 1.41%, ash 0.45%, fiber 2.00% (producers' data in dry mass). Fresh lucerne sprouts purchased from Uniflora Sp. z o. o. (Częstochowa, Poland) were used in amounts of 10, 20 and 30% of the total blend mass as replacement of flours in the recipe. Fresh lucerne sprouts (basic composition: moisture 92.10%, protein 3.99%, fat 0.69%, ash 0.40%, fiber 2.50%, producers' data in edible sprouts) were ground to a particle size lower than 1 mm by using a kitchen blender to create a pulp. Dry components were mixed and fresh lucerne sprouts pulp was added w/w and mixed with dry blend to create a homogenous mass. The mixed blends were tested for moisture content, and samples were moistened up to desired moisture level of 32, 34 and 36% of water content by the addition of a proper amount of tap water [Lisiecka and Wójtowicz 2020]. Blends were then manually transferred into the extruder hopper. Snack pellets were extruded with a single screw prototype modular extruder EXP-45–32 [built by Zamak Mercator, Skawina, Poland] with barrel configuration of L/D=20. The samples without additive (as control) and the samples supplemented with fresh lucerne sprouts pulp were processed at temperatures in individual sequential extruder sections: dosing section – 80 to 85°C, plasticizing section – 90 to 105°C, cooling section – 50 to 65°C, forming die – 60 to 75°C. Two variable screw speeds were applied: 60 and 100 rpm. The extruded material was shaped with a flat forming die of 0.6 mm x 30 mm as a strip, followed by an external cutting device to create tetragon samples of 30 x 30 mm. The cut snack pellets were dried at room temperature for 24 h (final moisture content below 11%) and then stored in hermetically sealed plastic bags.

Calculation of water consumption

The moisture content of ingredients was tested utilizing an air-oven by following the AACC 44–15.02 standard procedure [Kasprzycka et al., 2018]. Calculation of the water amount needed to crerate the desired initial moisture content of blends was based on the following formula (1) [Lisiecka and Wójtowicz, 2020]:

$$W = m_s \left(\frac{M_f - M_i}{100 - M_f} \right) \quad (1)$$

where: W – demand of water (kg/kg),
m_s – mass of sample (kg),
M_f – final moisture content of blend (%)
M_i – initial moisture content of blend (%).

The difference between the water amount needed to moisten the dry components and that needed for blends with fresh vegetable addition was calculated.

Processing efficiency and energy consumption

The processing efficiency (Q) was determined as a ratio of the obtained pellets mass at the defined time. The process efficiency was calculated using the formula proposed by Matysiak et al. [2018]:

$$Q = \frac{m}{t} \text{ (kg/h)} \quad (2)$$

where: Q – process efficiency (kg/h),
m – mass of fed extrudate (kg),
t – measurement time (h).

The measurement was repeated three times for each trial, the arithmetic mean of the measurements was adopted as the result.

The measurement of the extruder active power was recorded by a measuring system installed in the machine. The value of energy was converted into the specific mechanical energy (SME) using the formula provided by Matysiak et al. [2018]:

$$SME = \frac{n}{n_m} \times \frac{O}{100} \times \frac{P}{Q} \text{ (kWh/kg)} \quad (3)$$

where: SME – specific mechanical energy (kWh/kg),
n – extruder rotational speed measured with a tachometer (s),
n_m – extruder nominal speed measured with a tachometer (s),
O – extruder motor load versus maximum load (%),
P – nominal power indicated from control panel (kW),
Q – process efficiency described above (kg/h).

Phenolics content and antioxidant activity

The extracts from ground supplemented snack pellets were prepared using ultrasound-assisted extraction (UAE) by means of an ultrasonic bath [Bandelin Electronic GmbH & Co. KG, Berlin, Germany] at 60°C. In the first step 4 g of ground snack pellet were mixed with 80% ethanol (80 mL) and extracted (40 min). The extraction was carried out in two identical cycles. Both parts of extracts were combined, evaporated and dissolved in methanol (10 mL). The measurement of total content of polyphenolic compounds (TPC) was performed by applying the Folin-Ciocâlteu (FC) method (modified) [Burda and Oleszek, 2001]. In the initial step, 200 µL of extract was mixed with 1.8 mL of distilled water. Afterwards, 200 µL of FC reagent was added. After mixing the mixture was left for 5 min. Subsequently, 2 mL of 7% Na_2CO_3 was added and then incubated (for 60 min at 40°C). Absorbance was measured with the use of UV-VIS spectrophotometer (Genesys 20 UV-VIS, Thermo Scientific, Waltham, MA, US) at 760 nm. The level of phenolic compounds was expressed as µg gallic acid equivalents (GAE) per g of dry mass (d.m.). The free radicals scavenging activity of the obtained extracts was determined spectrophotometrically by using the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) method based on a method of Burda and Oleszek [2001]. The following parameters were applied: 517 nm wavelength, measurements every 5 min. for 30 min. The results are expressed as Trolox equivalent antioxidant activity (TEAC) calculated at 15 minutes from reaction initiation for all samples, and the TEAC values were calculated on the basis of a standard curve: $y = -0.0214x + 0.471$.

Water absorption and water solubility index

Water absorption index (WAI) and water solubility index (WSI) were assessed according to the methods described by Lisiecka et al. [2021] in triplicate. The ratio of the gel weight to the dry sample weight was defined as WAI. The ratio of the weight of dry solids in the supernatant to the weight of the dry sample was derived as WSI.

Statistical analysis

The obtained results were subjected to statistical analysis using the Excel software to calculate means of the results and standard deviations of multiple measurements.

RESULTS AND DISCUSSION

Calculation of water consumption

Dry components as wheat flour and corn flour applied in the control recipe (50/50) were characterized by the moisture content of 10.64%, whereas fresh lucerne sprouts showed 92.10% of moisture content. The initial moisture content of raw materials for snack pellets processing should vary from 25 to 36%, required to proper gelatinization of starchy components under the extrusion-cooking conditions used in the experiment [Matysiak et al., 2018; Lisiecka and Wójtowicz, 2020].

In the experiment, three levels of initial moisture were applied at levels of 32, 34 and 36%. Each blend, both control one and supplemented with fresh lucerne sprouts have to be moistened before treatment, so proper amount of technological water added must be calculated based on the equation (1). The results of the technological water added to each tested mixture are presented in Figure 1. Replacement of wheat-corn flour blends by fresh lucerne sprouts at various levels (10, 20 and 30%) allowed sufficient reduction of technological water which is needed in the extrusion-cooking process of snacks pellets. If control sample was moistened, at least 314 to 396 mL of water per kg of dry components need to be added to reach 32 and 36% of raw material blends moisture before the extrusion, respectively. Application of 10% of fresh lucerne sprouts enabled to limit water added to 201–258 mL per kg of dry components and for 20% of fresh additive the water amount was limited to 101–170 mL per kg, respectively to obtain 32 and 36% of initial moisture of components before the extrusion process.

The most significant water saving was reached when 30% of fresh lucerne sprouts were applied and when initial moisture was set at 32%, because in this case any additional technological water was needed. Similarly, only 10 mL per kg and 42 mL per kg was needed in the samples supplemented with 30% of fresh lucerne sprouts if the initial moisture of blends was set to 34 and 36%, respectively. Therefore, the limitation of water was from 89 to 100% if fresh lucerne sprouts were used, depending on the recipe and dough moisture level tested.

Looking for these numbers obtained in the laboratory scale, it seems not much but on the industrial scale, when the processing line efficiency is even up to 1000 kg per h, these savings may be very important both for producers, make the product easier to prepare, and for environment by limiting or completely avoiding of supply the technological water into processing of supplemented snack pellets. With the production capacity of 1000 kg/h, the water needed for the control recipe should be 396 kg/h if 36% of initial moisture is set. With daily work of commercial processing line from 10 to 12 hours, the water amount is 3960–4752 L daily. Dry starchy components used in the recipe were characterized the moisture content of 11.40% for wheat flour and 9.88% for corn flour, so the control mixture was 10.64% of initial moisture content in blend (50/50 wheat and corn flour). To obtain snack pellets with proper quality, the technological water must be supplied to reach the desired moisture levels from 25 to 36% [Panak Balentić et al., 2018].

Replacement of technological water in snack pellets composition by the natural water coming from fresh lucerne sprouts (92.10% of moisture) made it possible to obtain well-processed snack pellets without any addition of water, which was confirmed by the proper structure and stability of products, so daily limitation is possible from 4 to almost 5 m^3 of technological water, saving the environment and the money. This developed solution to replace the starchy components by fresh lucerne sprouts seems to be also beneficial due to the increasing amount of nutritionally valuable component from lucerne sprouts in final pellets, which would be confirmed by the analysis of nutritional characteristics of the obtained snack pellets.

Similar tendencies were reported by Lisiecka and Wójtowicz [2020], they found the possibility

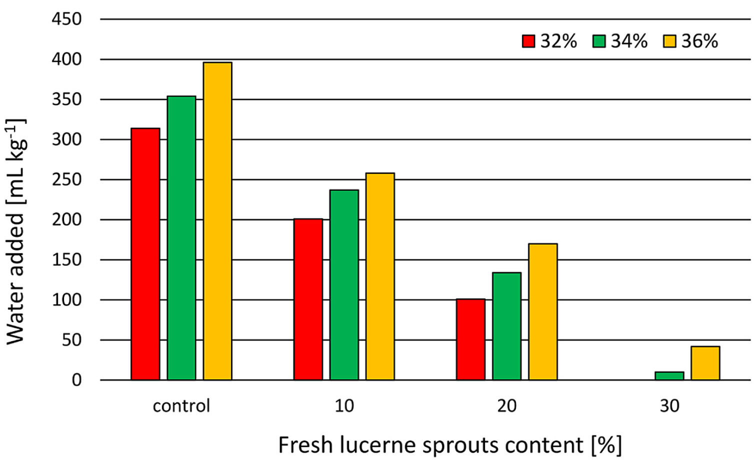

Figure 1. Water amount needed to reach the initial moisture of snacks pellets supplemented with 10, 20 and 30% of fresh lucerne sprouts

to save the water and energy by the addition of fresh vegetables into potato-based snack pellets. They reported the possibility to limit the technological water addition coming from fresh vegetables as the moisture content of fresh vegetable pulps was as follows: carrot–87.10%, kale–85.89%, leek–88.62% and onion–87.35%. Therefore, the water amount needed to moisten the dough for pellets to 33% of initial moisture content allowed limiting at least 0.334 kg water per kg of raw materials if 30% of additives were used. Thus, the use of vegetable additives in the amount of 30% reduced the amount of water consumed in the range from 97.92% (kale) to 99.5% (leek).

According to the European Commission (EC) Council Directive 96/61/EC on Integrated Pollution Prevention and Control (IPPC), the strategy to save the water is strongly advisable, especially as part of the Food Industry Sustainability Strategy (FISS) prepared by the Department of Environment, Food and Rural Affairs [DEFRA, London, UK]. They recommended as mandatory a 10–15% reduction of water in food industry by 2020, but there is no information if these recommendations have been fixed. The application of water efficiency management systems or wastewater re-use after treatment make it possible to save 30–50% of fresh water in industry [Nikmaram and Rosentrater, 2019].

This water saving is important to producers, so many activities were done to limit the technological water needs in food industry, such as dry peeling instead of wet peeling of fruits and vegetables, high pressure-low volume cleanup systems, dry pre-cleaning prior to washing, or optimization of water flow by flow restrictors [Ölmez, 2013]. Advisable reduction of fresh water in food industry should be up to 90%, so the finding presented in this paper completely fulfill these requirement and can be recommended directly to the producers as an environmentally friendly technological solution ready to implement on an industrial scale.

Processing efficiency and energy consumption

The efficiency of the extrusion-cooking process depends on many factors, including the raw materials used, their moisture content, granulation, functional additives, extrusion process temperature and extruder screw speed. The production of snack pellets using the addition of fresh lucerne sprouts allowed the development of technological guidelines for a new range of snack products. The addition of fresh lucerne sprouts to the raw material blends showed a differential effect on process efficiency (Table 1). The efficiency of the extrusion process mostly depended on the screw speed of the extruder, the sprout content in the mixture and initial moisture content of components. The highest extrusion process efficiency was observed for the blends with an initial moisture content of 32% extruded at the screw speed of 100 rpm. Matysiak et al. [2018] showed similar trends. The applied functional additive in the form of fresh lucerne sprouts had a significant effect on the extrusion process efficiency. The addition of fresh vegetables influences the physicochemical composition of the raw material mixtures, which directly translates into the extrusion process efficiency. The highest process efficiency (32.72 kg/h) was observed for the raw material blends containing 10% added fresh lucerne sprouts. Lisicka and Wójtowicz (2019) conducted an extrusion study of corn snacks and obtained an extrusion process efficiency of 32–36 kg/h. This may be due to the lower amount of water in the raw material mixture, which directly influences the flow speed of the mass inside the extruder.

The lowest extrusion efficiency (9.12 kg/h) was observed for the blends produced at 60 rpm of the extruder screw speed with the additive level of 30% and blend moisture content of 36%. The study also found that the extrusion efficiency is significantly influenced by the moisture content of the raw material mixture. This was confirmed by the results of the extrusion efficiency of the control sample without the addition of fresh lucerne sprouts. As the moisture content of the raw materials increased, the efficiency of the extrusion process decreased.

The results of SME was low as calculated per kg of product. The range of specific energy consumption varied from 0.035 to 0.179 kWh/kg. Higher SME values were found if greater initail moisture was applied during processing of snack pellets but the highest results were found for control wheat-corn snack pellets without additives. Increasing amount of fresh lucerne sprouts in the recipe lowered energy consumption which could be the effect of lower starch content and thus lower viscosity of the treated mass undergoing the gelatinization process [Prabha et al., 2012]. Also,

Table 1. Efficiency and energy consumption values obtained for new generation snack pellets fortified with fresh lucerne sprouts (data expressed as mean value of n = 3 ± SD standard deviation)

Fresh lucerne sprouts content (%)	Screw speed (rpm)	Moisture content (%)	Efficiency (kg/h)	SME (kWh/kg)
0	60	32	15.28 ± 0.54	0.086 ± 0.002
		34	27.81 ± 0.25	0.137 ± 0.002
		36	13.04 ± 0.32	0.142 ± 0.004
	100	32	31.44 ± 1.26	0.090 ± 0.003
		34	14.48 ± 1.46	0.137 ± 0.002
		36	25.36 ± 0.63	0.179 ± 0.005
10	60	32	20.80 ± 0.72	0.088 ± 0.003
		34	17.92 ± 2.01	0.090 ± 0.003
		36	16.48 ± 0.24	0.104 ± 0.002
	100	32	32.72 ± 0.98	0.045 ± 0.002
		34	20.72 ± 0.85	0.081 ± 0.004
		36	22.76 ± 0.56	0.061 ± 0.001
20	60	32	20.08 ± 0.72	0.035 ± 0.003
		34	17.84 ± 0.28	0.094 ± 0.003
		36	9.28 ± 0.24	0.181 ± 0.002
	100	32	27.84 ± 0.98	0.035 ± 0.002
		34	27.36 ± 2.01	0.061 ± 0.002
		36	22.76 ± 0.56	0.061 ± 0.001
30	60	32	15.36 ± 0.26	0.096 ± 0.002
		34	10.24 ± 0.28	0.153 ± 0.003
		36	9.12 ± 0.24	0.163 ± 0.004
	100	32	26.24 ± 0.84	0.068 ± 0.002
		34	22.01 ± 0.08	0.090 ± 0.004
		36	14.96 ± 0.60	0.118 ± 0.004

as compared to other snacks processess, the extrusion-cooking is less energy consuming, especially when semi or high-moisture conditions are applied. Meng et al. [2010] reported the SME increase along with screw speed and decreasing barrel temperature by applying twin screw extruder, but the feed moisture had no effect on SME (calculated SME varied from 87.9 to 115.8 Wh/kg).

Matysiak et al. (2018) reported higher energy consumption during the processing of wholegrain cereal raw materials than potato-based recipies, because the higher SME usually is the result the greater degree of starch gelatinization. Observations done by other authors suggesting that semi-moisture extrusion requires less energy, but as presented by da Silva et al. [2014] the SME values (ranged between 32.20 and 58.88 kJ/kg) of the extruded products processed under experimental conditions (moisture 11–21%, screw speed 333–378 rpm) showed that none of the variables studied (screw speed, moisture and bean level) significantly influenced the SME values.

Total content of polyphenolic compounds and free radical scavenging activity

An excellent method of improving the health and nutritional properties of plants is to germinate their seeds. The sprouts from early plant's growth cycle, so nutrient content is extremely high. Sprouts are a very rich source of bioactive compounds (e.g. polyphenols), minerals and vitamins.

Plants belonging to the genus *Medicago,* such as lucerne, were among the first cultivated by humans. This group of plants is characterized by having high nutritional properties, as well as enhanced vitamin (C, E, from group B, beta carotene) and mineral (potassium, calcium, magnesium, silicon, iron) composition. Additionally, due to the high level of chlorophyll, plants of the *Medicago* genus may have a detoxifying effect for human body [Krakowska-Sieprawska et al., 2021]. European Food Safety Authority accepted plants from this group as safe dietary ingredients. For now, their consumption by humans is still low, but there has been an increasing interest in using lucerne as additives for salads, supplements and functional food.

The contents of polyphenolic compounds and antioxidant properties of snack pellets enriched with *Medicago sativa* L. (lucerne, alfalfa) sprouts were tested in this paper to indicate the contained bioactive compounds and to establish their pro-health potential. The first stage of the analysis was to isolate the polyphenolic compounds contained within the pellets. Recent directions indicated by "green chemistry principles" in extraction methods have focused on the improvement of the efficiency of the process and reduction of solvent consumption [Krakowska-Sieprawska et al., 2021]. A recognized environmentally friendly technique is ultrasound-assisted extraction (UAE) with ethanol. UAE at elevated temperatures and with 80% ethanol was previously used by Kasprzak-Drozd et al. [2022] or Soja et al. [2023] to obtain polyphenolic extracts from the snacks enriched with vegetables and herbs. In these papers, UAE was demonstrated to be an optimal technique for extracting phenolics from a wide group of functional foods, therefore, it was applied in this experiment. The tested pellets with different quantities of lucerne sprouts (0, 10, 20, 30%) produced at various screw speeds and moisture contents by using extrusion-cooking showed high content of polyphenolic compounds increasing significantly due to the higher addition of lucerne sprouts (Table 2).

Results indicated the highest total content of polyphenols for the pellets with 30% addition of the lucerne sprouts processed at screw speed 60 rpm and at moisture content 36%, while the lowest was seen in a control sample without such functional additive that was extruded at the screw speed of 60 rpm and moisture content at 36%. Significant differences in the level of polyphenolic compounds in the produced snack pellets due to the effect of screw rotational speed and initial moisture content were observed. However, the discovered proportional increase in the level of polyphenolic compounds indicates the fact that extrusion technique did not degrade the active compounds present in pellets enhanced with *M. sativa* sprouts. This process may reduce the content of bioactive substances in the tested

Table 2. Total phenolic content and TEAC values obtained for new generation snack pellets fortified with fresh lucerne sprouts (data expressed as mean value of n=3 ± SD standard deviation)

Fresh lucerne sprouts content (%)	Screw speed (rpm)	Moisture content (%)	TPC content (µg GAE /g d.w. product)	TEAC value (µg/g d.w. product)
0	60	32	62.10 ±1.20	111.45 ± 4.38
		34	61.23 ± 1.45	112.21 ± 5.26
		36	60.60 ±1.90	113.08 ± 5.12
	100	32	78.10 ± 2.10	106.54 ± 6.11
		34	71.25 ± 1.98	102.78 ± 5.14
		36	60.80 ± 1.80	103.50 ± 4.98
10	60	32	102.10 ±7.62	117.76 ± 5.09
		34	104.23 ± 6.14	112.58 ± 4.48
		36	106.30 ± 5.12	116.12 ± 5.23
	100	32	105.10 ± 5.16	113.32 ± 4.99
		34	106.12 ± 4.69	118.45 ± 3.68
		36	107.70 ± 4.87	125.12 ± 5.89
20	60	32	135.12 ± 6.24	114.25 ± 5.12
		34	141.14 ± 7.12	116.85 ± 6.98
		36	139.24 ± 5.47	128.45 ± 8.02
	100	32	139.26 ± 4.89	121.21 ± 8.47
		34	146.69 ± 6.98	129.31 ± 6.51
		36	142.44 ± 6.47	126.98 ± 3.98
30	60	32	170.70 ± 7.56	126.92 ± 6.45
		34	175.23 ± 9.11	129.71 ± 8.11
		36	175.10 ± 7.12	126.75 ± 6.22
	100	32	170.70 ± 6.98	117.29 ± 5.98
		34	168.45 ± 7.12	121.65 ± 3.99
		36	169.50 ± 8.02	126.17 ± 4.98

Note: TPC – total phenolics content; TEAC – Trolox equivalent antioxidant activity; $^{a-d}$ – data in columns with similar letters did not differ significantly at α=0.05 by Tukey post-hoc test.

samples, but simultaneously can increase their bioavailability to the human body. Chiriac et al. [2020], for example, identified a total of 59 polyphenols, e.g. fenolic acids, isoflavones, flavones, flavonones, and flavonols in alfalfa sprouts. After analyzing the presented data, sprouts of the third day of germination were used in the conducted experiment. The biological actions of *Medicago* may be due to the presence of polyphenols and the function as free radical scavengers and/or metal chelators [Krakowska-Sieprawska et al., 2021]. Therefore, the DPPH free radical scavenging potential of the pellets was analyzed. The obtained results presented as Trolox equivalent antioxidant activity (TEAC, Table 2) confirmed that the antioxidant properties of products increased with the addition of fresh lucerne sprouts.

The highest antiradical properties were found in the pellets supplemented with 30% of lucerne sprouts produced using 60 rpm and with either 34 and 36% of moisture content. In general, the samples extruded at 60 rpm had higher antioxidant activity than those produced at 100 rpm. It is possible that the shear forces prevailing at 100 rpm may inactivate a part of the antioxidant compounds present in the pellets [Kasprzak-Drozd et al., 2022]. However, upon noticing slight differences in TEAC for both rotational speeds, this process must occur only to a small extent. Moreover, the increasing moisture content had a positive effect upon the antioxidant properties of snack pellets processed at 100 rpm. Thus, the moisture content of snack pellets can have an impact on their antioxidant properties; high moisture can prevent the degradation of the antioxidants, reducing shear forces inside the extruder. When analyzing the obtained study results, it was noted that both 34% and 36% moisture content did not have negative influence on the health-promoting activity if processed at 60 rpm. However, increase in rpm when lower (32%) moisture content was applied during processing caused more intensive shearing and thus lower antioxidant activity due to a higher shear stress. Hence, appropriate choice of selected production parameters may allow the protection of sensitive components affecting pro-health values of the extruded food. In the conducted study, the antioxidant activity of the snack pellets was positively correlated with the polyphenols content ($r = 0.81$). Polyphenols exhibit antioxidant activity by scavenging and neutralizing free radicals, which are highly reactive molecules that can cause oxidative stress and damage to cells. Therefore, the higher the polyphenol content in a snack pellet, the greater its potential to act as an antioxidant. It is important to note that the specific types and concentrations of polyphenols can vary among different snacks, depending on their ingredients and processing methods. Considering the positive correlation between polyphenol content and antioxidant activity, incorporation of ingredients rich in polyphenols, such as fruits, nuts, or whole grains, into snacks can enhance their antioxidant properties [Francis et al., 2022; Lotfi Shirazi et al., 2020; Lourenço et al., 2016; Soja et al., 2023].

WAI and WSI of snack pellets

Water absorption index (WAI) indicates the ability of a treated component to absorb and hold external water and it is a measurement of the amount of gel formed by processed starch, protein and fibers after swelling. During the extrusion-cooking a starch gelatinization and protein denaturation occur depending on processing conditions [da Silva et al., 2014; Meng et al., 2010]. Gelatinized starch absorbs more water trapped in the materials, thus increasing the WAI values. When material is treated too intensively, the starch granules may be destroyed and lose their water absorption ability [Ruiz-Armenta et al., 2019]. During the extrusion process, starch gelatinization is presented allowing a greater capacity of However; the presence of proteins or fibers in the extrudate composition may have effect on WAI due to changes affecting the hydrophilic/hydrophobic balance of the processed components [Lourenço et al., 2016]. WSI is a particularly important parameter of extrusion-cooking intensity and allows predicting raw material behavior during processing. In the tested samples, low water absorption in control wheat-corn snack pellets was noted (3.15–4.43 1/g), but, at the same time, these samples showed the highest level of WSI (10.34–14.72%). All results are presented in Table 3. An increasing amount of lucerne sprouts significantly enhanced water absorption of the tested snack pellets supplemented with fresh sprouts, and more than twice intensive water absorption ability was evaluated if 30% of additive was used, as compared to the control sample.

In the conducted study, high WSI values indicated that components were not sufficiently integrated in the pellet structure. This outcome was probably due to the high content of fiber and the low amount of starch needed to stick together all the components supplemented with fresh lucerne sprouts. The fiber fractions which are non-processable at low temperature can, however, be easily rinsed out from the snack pellet sample. Therefore, WSI was low in snack pellets with 10% of sprouts, but the lowest value was obtained for sample with 30% of lucerne processed at 60 rpm and 32% of initial moisture. Indeed, in all samples processed with these settings (32% m.c., 60 rpm) WSI was the lowest. Therefore, an improving effect of lucerne sprout addition on the WAI of snack pellets was perceived. What is more, processing conditions showed similar effects on the WAI and WSI of the tested samples. The higher WAI and WSI were observed when increased initial moisture content was applied during snack pellet processing. Similarly, higher initial moisture influenced increased WSI values for the products of both 60 and 100 rpm processing screw speeds. As WSI increased with increasing moisture and with higher screw speeds, this outcome indicates that the components were not sufficiently integrated in the pellet structure. This was probably due to the enhanced content of protein, fiber and other healthy components present in fresh sprouts, and thus lowered amount of starch in the recipes.

During the extrusion-cooking, due to the presence of water, heat and shear-forces the reactions between proteins, amylose, and amylopectin generate several changes that lead to reduction of WAI. In the research of Lisiecka et al. [2021] a negative correlation was demonstrated between WAI value and protein content, soluble dietary fiber content and ash content of the snacks supplemented with fresh beetroot pulp as a starch substitution. They found that extrusion-cooking induced an increase in the insoluble dietary fiber content and WAI in the obtained potato-based pellets.

Table 3. Results of WAI and WSI in new generation snack pellets fortified with fresh lucerne sprouts (data expressed as mean value of n=3 ± SD standard deviation)

Fresh lucerne sprouts content (%)	Screw speed (rpm)	Moisture content (%)	WAI (1/g)	WSI (%)
0	60	32	3.15 ± 0.03	10.34 ± 0.07
		34	3.64 ± 0.04	12.17 ± 0.06
		36	3.82 ± 0.05	14.14 ± 0.13
	100	32	3.43 ± 0.01	12.47 ± 0.43
		34	3.99 ± 0.02	13.47 ± 0.32
		36	4.43 ± 0.14	14.72 ± 0.05
10	60	32	3.76 ± 0.03	8.96 ± 0.01
		34	4.01 ± 0.02	9.12 ± 0.05
		36	4.12 ± 0.01	9.87 ± 0.08
	100	32	3.95 ± 0.03	9.03 ± 0.01
		34	4.16 ± 0.05	10.11 ± 0.02
		36	4.39 ± 0.12	10.84 ± 0.04
20	60	32	5.21 ± 0.02	9.28 ± 0.04
		34	5.01 ± 0.04	10.11 ± 0.06
		36	5.26 ± 0.02	11.14 ± 0.06
	100	32	6.52 ± 0.12	10.13 ± 0.12
		34	6.21 ± 0.19	9.24 ± 0.24
		36	6.98 ± 0.14	10.31 ± 0.15
30	60	32	6.29 ± 0.04	7.78 ± 0.04
		34	6.98 ± 0.03	8.58 ± 0.07
		36	7.08 ± 0.13	10.46 ± 0.11
	100	32	6.79 ± 0.11	11.93 ± 0.15
		34	7.12 ± 0.04	12.23 ± 0.41
		36	7.46 ± 0.05	13.07 ± 0.20

Note: WAI – water absorption index; WSI – water solubility index; $^{a-j}$ – data in columns with similar letters did not differ significantly at α=0.05 by Tukey post-hoc test.

After processing the material in the proposed process conditions, higher WAI and WSI index were recorded for the obtained beetroot-supplemented pellets in relation to the unprocessed control sample. The maximum WSI value of 8.63% was determined for the pellets with 10% of fresh beet pulp extruded at 80 rpm, but the lowest value of the WSI index was 1.42% for the pellets from the control potato sample produced at similar screw speed. It was concluded in the work that very low solubility may be the effect of lower starch gelatinization temperature of potato components compared to wheat and corn raw materials.

Ruiz-Armenta et al. [2019] observed an increase in WSI after extrusion in corn starch-based pellets supplemented with naranjita and milk powder. They held the opinion that this effect was linked to the loss of the semicrystalline form of native starch granules after processing. In their study, the maximum WAI value of pellets and snacks with carrot pulp addition was 4.90 1/g when 5% of carrot pulp was added during the extrusion at 80 rpm. However, significant differences in WAI were observed in the pellets with the maximum content (30%) of fresh carrot pulp in the composition, as compared to the control sample extruded at the same speed of the extruder screw.

In the work, minimal WSI was found to be 1.42% in snack pellets with 5% of vegetable additive produced at the minimum rotational speed of the extruder screw. Ruiz-Armenta et al. [2019] noted that the WAI of pellets was negatively correlated with soluble fiber and the WSI of pellets was positively correlated with the content of protein, ash, soluble and total fiber, and negatively with fat content. Lisiecka et al. [2021] tested snack pellets with the addition of fresh vegetables of *Allium* genus and they discovered that WAI ranged from 3.04 g/g in pellets with 20% of added leek pulp, extruded at 100 rpm, to 4.65 g/g, in the pellets with 2.5% of leek additive content produced at the highest screw speed, while the WSI values were determined in the range from 0.20% (pellets with the highest level of WAI) to 7.48% (pellets with content of 2.5% of onion pulp produced at 60 rpm). The statistical analysis confirmed that the level of fresh vegetable pulp had a significant negative impact on the WAI and WSI values of all pellet products.

CONCLUSIONS

Replacement of wheat-corn flour blends by fresh lucerne sprouts at various levels (10, 20 and 30%) allowed sufficient reduction of technological water which is needed in the extrusion-cooking process of snacks pellets. The limitation of water was from 89 to 100% if fresh lucerne sprouts were used, depending on the recipe and dough moisture level tested. Replacement of technological water in the composition of snack pellets by the natural water coming from fresh lucerne sprouts (92.1% of moisture) made it possible to obtain well processed snack pellets, which was confirmed by proper structure and stability of products, without any technological water added if 30% of lucerne sprouts were applied. In an industrial practice with high-capacity processing line, the daily saving from 4 to 5 m^3 of technological water may be done if this technology will be adopted. The addition of fresh lucerne sprouts had a significant impact on the nutritional quality of developed wheat-corn blend-based snack pellets. The almost triple increase of total phenolics content at 30% of additive was observed as compared to the base control sample. The addition of fresh lucerne sprouts to the basic recipe at the level of 10, 20 and 30% also had significant effect on the snack pellets WAI and WSI. Moreover, WAI increased if the initial moisture level was higher and if higher screw speed was applied during snack pellets extrusion. On the basis of the presented results, 36% initial moisture content and 60 rpm screw speed were recommend as the appropriate processing conditions to produce acceptable lucerne sprout fortified snack pellets for the market. However, the developed snack pellets are only semi-finished products, so further research is needed in terms of final expansion energy requirements depend on the expansion method used and sensory analysis and consumer acceptance before the production of a ready-to-eat expanded snack that contains lucerne for the market.

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