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Lucerne (*Medicago sativa* L.) valorization in a gluten free pastry product

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Abstract

Lucerne (*Medicago sativa* L.) is a sustainable perennial crop with a small impact on the environment resources but with a big importance from the nutritional standpoint. It is a rich vegetable source of protein, minerals, total phenols, phenolic acid, carotenoids, and vitamins. In the present work, the main purpose was to obtain a gluten free product by replacing almond flour with lucerne powder in different addition such as 3%, 6% and 9%, respectively. Chemical analysis showed that protein, ash, moisture, total phenols and antioxidant activity increased as the percentages of alfalfa increased. For instance, total phenols reached a value of 44.67 mg gallic acid/100g fresh weight at an addition of 9% alfalfa, whilst sample with 3% addition registered a value of only 36.21 mg gallic acid/100g fresh weight. Sensorial analysis showed that the optimum addition of alfalfa is 6%, having a final hedonic score of 8.62.

Keywords: lucerne *(Medicago sativa L)*, sustainability, sensory evaluation, chemical composition

1.Introduction

Lucerne (*Medicago sativa* L.) also known as alfalfa, is a perennial legume mainly used as animal feed with several environmental and agronomic advantages such as protection of soil erosion, preservation of soil fertility and reduction of greenhouse gas emissions [1]. Furthermore, this crop is able to face rainfall deficiencies and could successfully contribute to the biological nitrogen fixation [2] thanks to its roots colonized by bacteria able to fix nitrogen from the air (Figure 1). Moreover, lucerne could be harvested several times/year and could have phytobiotic activity in humans and animals [3].

Lucerne (*Medicago sativa* L.) is an ancient plant with nutritional implications in folk medicine in countries such as America, Turkey but also Iraq. It has been used in the treatment and prevention of several diseases such as ulcers, bloats, different digestive tract diseases, but also for appetite stimulation, or even to increase the resistance of immune human system [3]. From a nutritional standpoint, lucerne is a rich source in carbohydrates, proteins, minerals, vitamins and polyphenols [4].

It is also important to mentioned its rich composition in amino-acids such as valine, lysine, leucine or even threonine, minerals such as Ca, Si, Fe, Mn, Cu, Mg, P and vitamins (B1, B12, B6, B2, C, folic acid, inositol, choline), as reported by [5]. Likewise, [6] mentioned that alfalfa had a chemical composition rich in phytoestrogens such as lignans, isoflavones and coumestans which are considered biomolecules with important implications in the treatment and prevention of diseases such as cardiovascular ones, osteoporosis, therapy and chemoprevention of cancer. Other pharmaceutical effects of lucerne could be the prevention of anemia, endometriosis, diabetes, arthritis, asthma and different nervous system disorders [6].

Due to its high nutritional content is has been called in the ancient times as the *father of all food* and today it is also entitled as the *queen of forages*. EFSA (European Food Safety Authority) already accepted alfalfa leaf extract to be used as a supplement rich in vitamins and minerals [5], meantime, alfalfa seeds and herb are considered as GRAS (*Generally Recognized as Safe*), [4].

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In human nutrition, lucerne (*Medicago sativa* L.) is already included in some nutritional supplement such as powder (Figure 2), capsules or tablets manufactured from the isolated plant protein or as a food in salads, soups, puddings or even tortillas and croquets [7]. In Europe, alfalfa is mainly consumed such as sprouts raw or cooked in sandwiches or salads [8].

On the other hand, gluten free products manufacturing is quite difficult mainly because of the lack of gluten. Therefore, from the technological point of view, in order to have similar gluten free products with the conventional ones, the use of hydrocolloids or starches is indispensable. Agar-agar is considered as a hydrocolloid able to mimic the rheological properties of gluten such as viscosity and therefore, improving the textural characteristics of the final baked goods [9]. Another problem of gluten free products is their human nutritional implication, being poor in minerals, vitamins and rich in salt and sugar [10]. Therein, there is still a need on the market for developing new gluten free products able to cover the nutritional human needs.

Therefore, the main goal of the present research is to valorize alfalfa powder in the manufacturing of a gluten free tender dough pastry product such as tart.

Figure 1. Lucerne (*Medicago sativa* L.) plant

Figure 2. Lucerne (*Medicago sativa* L.) powder

2. Materials and Method

2.1. Materials

The raw materials used in the present research were purchased from specialized local stores from Cluj-Napoca. All the experiment was carried out in the bakery pilot station and laboratories from the Faculty of Food Science and Technology, Cluj-Napoca, University of Agricultural Scienceas and Veterinary Medicine.

2.2. Gluten free Tart tender dough manufacturing

The almond flour was replaced with different percentages of alfalfa powder, as follows: 3%, 6% and 9%, respectively (Table 1). Oatmeal, almond flour, and alfalfa powder were mixed together. On the other hand, vanilla soy milk was homogenized with agar-agar in order to obtain a hydrated final mix. All the ingredients were homogenized and stored at 4°C before being divided and molded in the final forms. The baking process was realized in a Zannoli oven at 180°C for 15 minutes.

Table 1. Gluten free tart manufacturing

Raw materials, g	**Almond flour and lucerne powder addition**			
Almond flour	100	97	94	91
Lucerne powder	0	3	6	9
Vanilla soy milk	50			
Oatmeal	12			
Agar-agar	2			

2.3. Physico-chemical characterization of the final baked goods

AACC methods were used for moisture (AACC 44-15.03), total ash (08-01.01), lipid (30-25.01), and protein (46-11.02) analysis. Color coordinates such as *l** (lightness*)*, *a** (red/green coordinate*)*, *b** (yellow/blue coordinate), were analyzed by using an NH 300 portable colorimeter (Shenzhen Threenh Technology Co.,Ltd., Shenzhen, China), as described by our research group [6].

Total phenols were analyzed according to the method described by our research group [11]. Briefly, 100 µl of methanolic extract was mixed with Folin Ciocalteu (500 µl) reagent, 2 ml of 7.5% Na2CO3 and distilled water to brought up the final volume to 10 ml. The obtained solutions were kept for 120 minutes in the dark and measured at a wavelength of 750 nm with a Schimadzu 1700 spectrophotometer (Schimadzu Scienctific Intruments, Kyoto, Japan). DPPH (1,1-Diphenyl-2-picrylhydrazyl) reagent was used for antioxidant samples analysis. Shortly, 100 µl of methanolic extract was mixed with 3.9 ml DPPH and kept in the dark for 30 min. The samples absorbance was read at 515 nm and calculated as the ratio between the difference of DPPH absorbance and samples absorbance, divided by DPPH absorbance solution and multiplied by 100.

Sensory analysis was carried out based on nine hedonic scale, as described by [12]. Sensorial parameters such as taste, texture, overall acceptability and color were analyzed by 30 panelists, students and staff from the faculty of Food Science and Technology.

2.4. Statistical analysis

Data obtained were statistically analyzed by using Duncan multiple comparation test, according to our research [13].

3. Results and Discussion

3.1. Chemical analysis of the obtained final baked products

Alfalfa addition in 3%, 6% and 9 % percentages influenced in a positive way parameters such as moisture, ash, protein, total phenols, and antioxidant activity, as displayed in Table 2. Moisture content is mainly influenced by the addition of agar-agar which is a hydrocolloid with water retention properties thanks to its hydrophilic nature [14]. Furthermore, the fiber content of the ingredients together with their granularity could influenced the water absorption capacity, as emphasized in other study by [15].

The increased ash content could be explained by the addition of alfalfa powder, being a rich source in minerals. In line with this, [16] showed that alfalfa mineral content could be influenced by the pedo-climatic condition. For instance, Na was identified in arrange of 201 to 285 mg/kg dry matter, Ca in a range of 45 546-53 060 mg/kg dry matter, meantime, Fe and Cu registered values between 386 and 647 mg/kg dry matter and 4-5 mg/kg dry matter, respectively.

With respect to protein and fat content, addition of alfalfa increased the protein content from 18.67% for the control sample to 19.03% at an addition of 9%, highlighting that alfalfa powder could be considered also a rich source of protein.

Likewise, recently, [17] showed that alfalfa is an important source protein with a value of 204 g/kg dry matter, whilst, [4] reported a value between 5.20-20.4%. it is worth to pointing out that alfalfa flour had significant content of essential amino acids, such as leucine (6.42 g/100g protein), phenylalanine (4.12 g/100g protein), valine (4.25 g/100g protein), isoleucine (3.23 g/100g protein), lysine (3.46 g/100g protein) and threonine (3.59 g/100g protein),[8]. According to [4] fat content could varied between 0.9-2.6% depending on the maturity of the lucerne, whilst, reported an amount of 1.39 g/100g dry weight [8].

Color parameters such as *L**, *a**, *b**, showed that *L** and *b** coordinates decreased as increased the lucerne powder addition due to its strong green color, whilst *a** value decreased, as showed in Figure 3. Color is an important characteristic that could highlighted the influence of temperature degradation during thermal treatments such as baking [6]. Moreover, Maillard reaction could also influenced the color of the final baked products, playing a major role in the color formation [18]. Nonetheless it is important to mentioned that also the presence of natural pigments such as polyphenols together with chlorophyl could influence the L* values of the baked goods [18].

Regarding the total phenols and antioxidant activity, addition of alfalfa increased both values, mainly because its rich chemical composition. [6] identified that addition of alfalfa powder in extrudates manufacturing successfully increased phenolic acid content such as caffeic acid, syringic, ferulic, Di-caffeoylquinic acid but also their antioxidant activity.

In line with this, [19] showed that mainly flavonoids such as flavonol, flavone, flavone-C-glycoside identified in alfalfa are responsible for its powerful antioxidant capacity. Likewise, [6] identified in alfalfa powder flavones such as L-diglucur (Luteolin-diglucuronide), A-ferr-gg (Apigenin-(feruloyl-glucuronyl)-glucuronide), Liq-gen (Liquirtigenin), but also isoflavones such as genistein. Moreover, [20] identified in different parts of alfalfa (*Medicago sativa* L.). liquiritigenin, hesperetin, eriodictyol, naringenin from flavones group

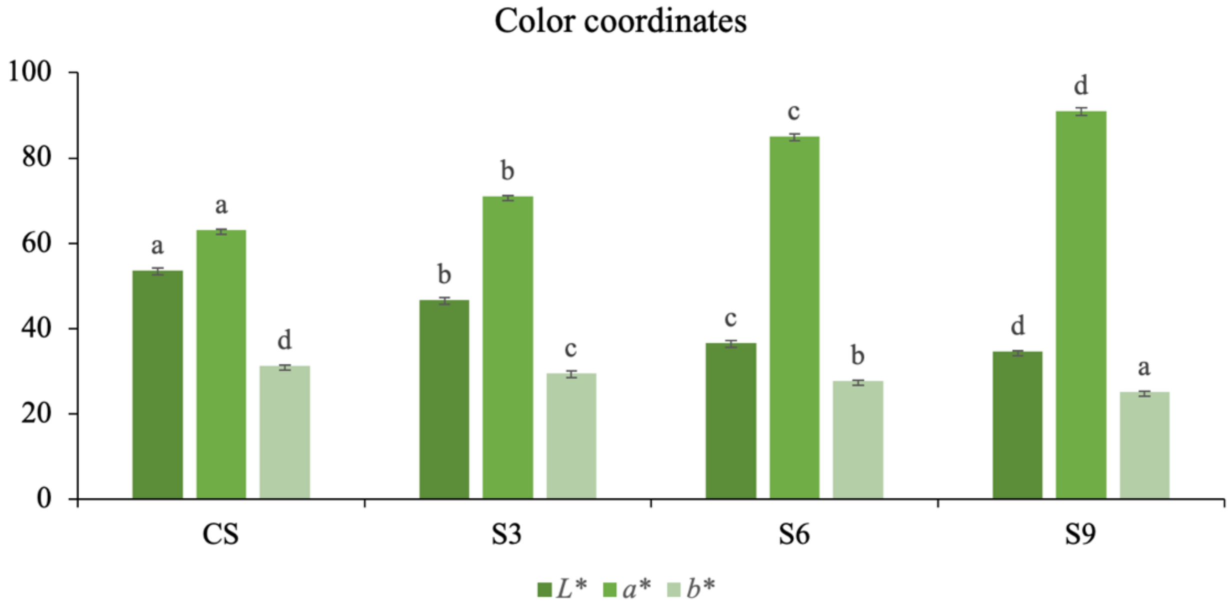

Figure 3. Color parameters of final baked products (*l** (lightness*)*, *a** (red/green coordinate*)*, *b** (yellow/blue coordinate)).

Table 2. Tart chemical analysis

Samples	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Total phenols (mg GAE/100g product)	Radical scavenging activity (%RSA)
CS	18.71 ± 0.05^{a}	2.19 ± 0.03^{a}	18.67± 0.05^{a}	56.55± 0.08^{a}	67.11± 0.27^{a}	31.09± 0.22^{a}
S3	18.02 ± 0.07^{ab}	2.39 ± 0.21^{ab}	18.71± 0.43^{ab}	56.50 ± 0.36^{ab}	70.50 ± 0.67^{b}	36.21 ± 0.05^{b}
S6	19.50 ± 0.23^{c}	2.90 ± 0.55^{c}	18.92 ± 0.17^{ab}	56.31± 0.18^{ab}	81.50 ± 0.44^{c}	39.64 ± 0.34^{c}
S9	20.00 ±0.43^{d}	3.35 ± 0.36^{d}	19.03 ± 0.09^{bc}	56.22± 0.36^{ab}	92.50 ± 0.31^{d}	44.67 ± 0.54^{d}

*CS = control sample, with 100% almond flour; S3= 97% almond flour +3 %lucerne powder; S6 = 94% almond flour +4 % lucerne powder; S9= 91% almond flour + 9% lucerne powder. *All analyses were made in triplicate and mean value ± standard deviation were recorded;*

** different small letters in a column means statistically significant differences between samples (p<0.05)

3.2. Sensorial analysis

The main analyzed attributes of the final products were visual appearance, overall acceptability, color, texture, flavor, taste and odour. Increasing alfalfa addition with 9% decreased mainly flavor, odor and taste characteristics, reaching final hedonic scores of 8.2, 7.5, and 7.0, respectively (Figure 2.). This could be explained probably by its grassy taste [4], supported by the aroma volatile compounds identified by [6] such as 1-hexanol, hexanal, nonanal, 2-octenal (E) and α -Thujene with odor perception of green, nut, leaf, herb or even wood. On the other hand, the highest hedonic score on the attribute of overall acceptability was displayed by the sample with 6% alfalfa powder addition.

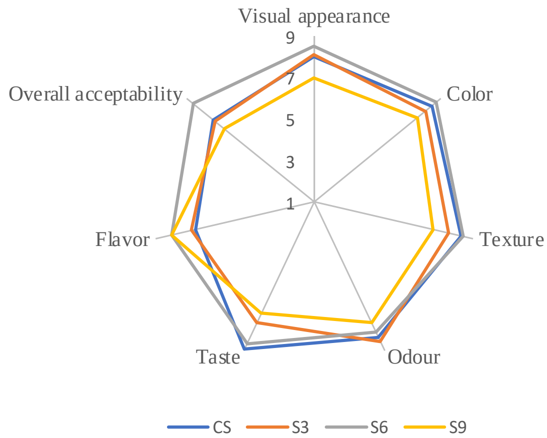

Figure 4. Sensorial analysis of the obtained products

4. Conclusions

Lucerne (*Medicago sativa* L.) powder is a rich source in bioactive compounds and a sustainable vegetable matrix due to its less negative influence on the environment, thanks to its ability to capture nitrogen from the air and its quite good adaptability to drought. In the present work, addition of 6% lucerne powder was the optimum percentage that increase protein, ash, total phenols, and antioxidant activity amounts, without negative effect on the consumers perception. Therein, 6% addition of lucerne powder could be successfully used in gluten free tart manufacturing process.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

References

1. Tucak, M.; Čupić, T.; Horvat, D.; Popović, S.; Krizmanić, G.; Ravlić, M. Variation of phytoestrogen content and major agronomic traits in alfalfa (medicago sativa l.) populations. *Agronomy* **2020**, *10*, doi:10.3390/agronomy10010087.
2. Viti, C.; Bellabarba, A.; Daghio, M.; Mengoni, A.; Mele, M.; Buccioni, A.; Pacini, G.C.; Bekki, A.; Azim, K.; Hafidi, M.; et al. Alfalfa for a sustainable ovine farming system: Proposed research for a new feeding strategy based on alfalfa and ecological leftovers in drought conditions. *Sustain.* **2021**, *13*, 1–13, doi:10.3390/su13073880.
3. Gaweł, E.; Grzelak, M.; Janyszek, M. Lucerne (Medicago sativa L.) in the human diet—Case reports and short reports. *J. Herb. Med.* **2017**, *10*, 8–16, doi:10.1016/j.hermed.2017.07.002.
4. Mielmann, A. The utilisation of lucerne: A review. *Br. Food J.* **2013**, *115*, 590–600, doi:10.1108/00070701311317865.
5. Rafińska, K.; Pomastowski, P.; Wrona, O.; Górecki, R.; Buszewski, B. Medicago sativa as a source of secondary metabolites for agriculture and pharmaceutical industry. *Phytochem. Lett.* **2017**, *20*, 520–539, doi:10.1016/j.phytol.2016.12.006.
6. Igual, M.; Chiş, M.S.; Socaci, S.A.; Vodnar, D.C.; Ranga, F.; Martínez-Monzó, J.; García-Segovia, P. Effect of Medicago sativa Addition on Physicochemical, Nutritional and Functional Characteristics of Corn Extrudates. *Foods* **2021**, *10*, 928, doi:10.3390/foods10050928.
7. Martínez, R.; Kapravelou, G.; Porres, J.M.; Melesio, A.M.; Heras, L.; Cantarero, S.; Gribble, F.M.; Parker, H.; Aranda, P.; López-Jurado, M. Medicago sativa L., a functional food to relieve hypertension and metabolic disorders in a spontaneously hypertensive rat model. *J. Funct. Foods* **2016**, *26*, 470–484, doi:10.1016/j.jff.2016.08.013.
8. Apostol, L.; Iorga, S., Mosoiu, C., Racoviță, R.C.; Niculae, O., M.; Vlasceanu, G. Alfalfa concentrate-a rich source of nutrients for use in food products, Journal of International Scientific Publications, **2017**, 5,666-73.
9. Bourekoua, H.; Różyło, R.; Benatallah, L.; Wójtowicz, A.; Łysiak, G.; Zidoune, M.N.; Sujak, A. Characteristics of gluten-free bread: quality improvement by the addition of starches/hydrocolloids and their combinations using a definitive screening design. *Eur. Food Res. Technol.* **2018**, *244*, 345–354, doi:10.1007/s00217-017-2960-9.
10. Chiş, M.S.; Păucean, A.; Man, S.M.; Vodnar, D.C.; Teleky, B.E.; Pop, C.R.; Stan, L.; Borsai, O.; Kadar, C.B.; Urcan, A.C.; et al. Quinoa sourdough fermented with *lactobacillus plantarum* ATCC 8014 designed for gluten-free muffins—a powerful tool to enhance bioactive compounds. *Appl. Sci.* **2020**, *10*, 1–23, doi:10.3390/app10207140.
11. Chiş, M.S.; Păucean, A.; Stan, L.; Mureşan, V.; Vlaic, R.A.; Man, S.; Muste, S. *Lactobacillus plantarum* ATCC 8014 in quinoa sourdough adaptability and antioxidant potential. **2018**, *23*, 13581–13591.
12. Man, S.M.; Păucean, A.; Călian, I.D.; Murean, V.; Chi, M.S.; Pop, A.; Murean, A.E.; Bota, M.; Muste, S. Influence of Fenugreek Flour (Trigonella foenum-graecum L.) Addition on the Technofunctional Properties of Dark Wheat Flour. *J. Food Qual.* **2019**, *2019*, doi:10.1155/2019/8635806.
13. Chis, M.S.; Păucean, A.; Man, S.M.; Bonta, V.; Pop, A.; Stan, L.; Beldean, B.V.; Pop, C.R.; Muresan, V.; Muste, S. foods Effect of Rice Flour Fermentation with Lactobacillus spicheri DSM 15429 on the Nutritional Features of Gluten-Free Muffns. *Foods* **2020**, *9*, 1–21.
14. Alvarenga, N.B.; Lidon, F.C.; Belga, E.; Motrena, P.; Guerreiro, S.; Carvalho, M.J.; Canada, J. Characterization of Gluten-free Bread Prepared From Maize, Rice and Tapioca Flours using the Hydrocolloid Seaweed Agar-Agar. *Recent Res. Sci. Technol.* **2011**, *3*, 64–68.
15. Man, S.; Păucean, A.; Muste, S.; Chiș, M.-S.; Pop, A.; Ianoş, I.-D.C. Assessment of amaranth flour utilization in cookies production and quality. **2017**, *23*, 97–103.

16. Gjoroska, V.B.; Krstik, M.; Gudeva, L.K. Determination of mineral composition in the alfalfa. **2019**, *17*.
17. Li, R.; Zheng, M.; Jiang, D.; Tian, P.; Zheng, M.; Xu, C. Replacing alfalfa with paper mulberry in total mixed ration silages: Effects on ensiling characteristics, protein degradation and in vitro digestibility. *Animals* **2021**, *11*, doi:10.3390/ani11051273.
18. Giuberti, G.; Rocchetti, G.; Sigolo, S.; Fortunati, P.; Lucini, L.; Gallo, A. Exploitation of alfalfa seed (*Medicago sativa* L.) flour into gluten-free rice cookies: Nutritional, antioxidant and quality characteristics. *Food Chem.* **2018**, *239*, 679–687, doi:10.1016/j.foodchem.2017.07.004.
19. Chen, S.; Li, X.; Liu, X.; Wang, N.; An, Q.; Ye, X.M.; Zhao, Z.T.; Zhao, M.; Han, Y.; Ouyang, K.H.; et al. Investigation of Chemical Composition, Antioxidant Activity, and the Effects of Alfalfa Flavonoids on Growth Performance. *Oxid. Med. Cell. Longev.* **2020**, *2020*, doi:10.1155/2020/8569237.
20. Kruk, J.; Baranowska, I.; Buszewski, B.; Bajkacz, S.; Kowalski, B.; Ligor, M. Flavonoids enantiomer distribution in different parts of goldenrod (Solidago virgaurea L.), lucerne (*Medicago sativa* L.) and phacelia (*Phacelia tanacetifolia* Benth.). *Chirality* **2019**, *31*, 138–149, doi:10.1002/chir.23041.