Changes in Selected Vitamins, Microorganism Counts, and Sensory Quality During Storage of Pressurised Sprouted Seed of Alfalfa (*Medicago sativa* L.)

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Abstract

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The aim of this study was to examine the changes of nutritional and sensory quality of sprouted alfalfa seed treated by high pressure, that take place during storage. Along with this, microbiological safety was also observed. Sprouted alfalfa seed in citric acid pickle, packed in transparent laminated bags PA/PE 80, was treated with 500 MPa high pressure for 10 minutes. The processed seed in bags was stored in a refrigerator for 21 days. The bags were sampled in regular intervals to perform analyses. The changes in the contents of vitamin C, riboflavin, niacin, and pantothenic acid were observed during storage. The same samples were also checked for microbiological safety and sensory quality. Vitamin C showed a significant decrease during storage. The content of vitamin C fell markedly after high pressure treatment (by 77%) and further decreased by 10–20% during storage. The values of riboflavin content did not change very much as a consequence of pressurisation or the storage period. The contents of niacin and pantothenic acid kept decreasing until the 3rd day of storage by some 60% in total and then remained unchanged. Sensory descriptors indicated quality decrease. High pressure treatment damaged the tissues of sprouted alfalfa seed which subsequently manifested itself particularly in the deterioration of appearance and texture quality. An additional overall impairment of the seed appearance and texture occurred during its storage. Microbiological safety of sprouted alfalfa seed was preserved throughout the storage time.

Keywords: sprouting; sprouted seeds; alfalfa; nutritional evaluation; sensory quality; microbiological evaluation; vitamins; high pressure treatment; storage; pressurisation

Sprouting of grains and seeds is one of the processing methods aimed at increasing the nutritive value of food in a natural way (Augustin & Klein 1989; Plaza *et al.* 2003). The process is simple and inexpensive to carry out. Different seeds can be sprouted for human consumption: all legumes (bean, pea, lentil, soybean), grains (rye, wheat, barley, oat), and seeds of other plants (alfalfa, radish). Sprouting seed significantly increases the levels of vitamins, minerals, and other important nutritional substances. Sprouting effects on the nutritional and bioactive factors of seeds

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or grains vary with the time of germination, light, moisture, temperature, and seed varieties.

Alfalfa is one of the oldest cultivated plants (Meyerowitz 1999). Originally it was called lucerne, or buffalo herb, the Arabic called this plant alfalfa. It means "Father of all foods". The Arabs started eating it after observing how it rejuvenated their grazing animals. Modern research shows that alfalfa also contains a considerable amount of therapeutic plant compounds, for example canavanine, an amino acid analogue. The sprouts are also rich in phytoestrogens, which have many functions similar to those of human estrogen but lack its adverse effects. Alfalfa sprouts are also a superb source of saponins and active antioxidants.

High hydrostatic pressure technology (pascalization) is considered as one of the prospective methods of nonthermal food preservation. High pressure treatment inactivates a number of enzymes and microorganisms contained in foods, whereas the sensory and nutritional properties remain practically unaltered (Hayashi 1989; Cheftel 1995). Pascalisation can extend the storage life of foods. Earlier authors found that high pressure causes only minor losses of group B vitamins in meat (Bognár *et al.* 1993; Mašková *et al.* 2000), and no significant losses of vitamin C were actually found in pressurised fruit juices (Kloczko & Radomski 1996). Sierra *et al.* (2000) did not find any losses either of group B vitamins in pressurised milk. However, any information on the behaviour of some vitamins during storage of pressurised food is still lacking.

This paper deals with the changes of selected vitamins (C, riboflavin, niacin, and pantothenic acid), microorganism counts, and sensory quality of pressurised sprouted alfalfa seed, as observed during storage.

MATERIAL AND METHODS

The input raw material used was sprouted alfalfa seed, having sprouted for 7 days, supplied by Beskyd Co. Fryčovice. It was poured over with aqueous citric acid pickle to have pH = 2 as the final value. Three parts of the pickle were used per two parts of sprouted alfalfa seed (w/w). Six 300 g samples and twelve 100 g samples contained in polyamide-polyethylene (PA/PE) bags were used. They were treated with 500 MPa pressure maintained for 10 min under ambient temperature, using the isostatic press ŽĎAS, a. s., type CYX, located in the Food Research Institute Prague (FRIP), having 2 litre chamber volume and maximum pressure 600 MPa.

Non-pressurised, pressurised, and pressurised and then stored sprouted alfalfa seed was then examined for the quality and food safety parameters. The samples were stored in a refrigerator at 4 ± 1°C. Sub-samples for analyses were taken on day 1, 3, 7, 14, and 21 following pressurisation. Further, non-pressurised sprouted seed kept without any pickle addition was subjected to microbiological evaluation. The acidity of the pickle was checked repeatedly during storage; it was always below pH 4.

Vitamin C – determined according to ČSN ISO, procedure No. 6557/2, method A.

Riboflavin – determined according to ČSN, procedure No. 56 0054.

Niacin – determined by the microbiological method using *Lactobacillus plantarum ATCC 8014*, ČSN procedure No. 56 0051.

Pantothenic acid – determined by the modified microbiological method using *Lactobacillus plantarum ATCC 8014*, ČSN procedure No. 56 0060.

Sensory evaluation – was performed in the specialised FRIP laboratory under the conditions set by ISO 6658 and 8589. The assessors focused on the changes in selected sensory descriptors as dependent on time and the way of preservation. The samples presented were evaluated by a panel of 12 trained judges who assessed their appearance, flavour, taste, acid taste, and texture using a scale method and recording the scores on the prepared forms with help of a non-structured graphic scale (Pokorný *et al.* 1999).

Microbiological evaluation – total microorganism counts (TMC) according to ČSN ISO 4833; *Escherichia coli* according to ČSN ISO 16649-2; yeasts according to ČSN ISO 7954; coliform bacteria according to ČSN ISO 4832; moulds according to ČSN ISO 7954.

RESULTS AND DISCUSSION

The nutritional evaluation of sprouted, high pressure treated alfalfa seed

Table 1 shows the dependence of the contents of the vitamins determined in pressurised alfalfa seed on the storage time. Non-pressurised sprouted alfalfa seed served as a control. The values found

Table 1. Contents of observed vitamins in non-pressurised, pressurised, and pressurised stored seed

Analyte		Non-pressurised	Pressurised day 1	Pressurised, stored (days)			
				3	7	14	21
Riboflavin	(mg/100 g)	0.062	0.062	0.044	0.046	0.055	0.052
	(mg/100 g DM)	1.25	1.22	0.94	0.87	1.06	1.32
Vitamin C	(mg/100 g)	3.22	0.75	0.51	0.28	< 0.20	< 0.20
	(mg/100 g DM)	64.8	14.8	10.8	5.3	< 3.8	< 5.1
Niacin	(mg/100 g)	0.3	0.3	0.2	0.1	0.1	0.2
	(mg/100 g DM)	5.5	4.9	2.1	2.2	2.3	2.2
Pantothenic acid	(mg/100 g)	0.36	0.33	0.17	0.15	0.16	0.16
	(mg/100 g DM)	6.01	5.57	2.43	2.38	2.48	2.27

in the control sample were in a good agreement with the data of Augustin and Klein (1989). No pronounced changes occurred with riboflavin; its content in mg/100 g dry matter was found slightly higher after 21 days of storage compared to the untreated control and to the pressurised seed stored for one day. Table 2 shows losses of selected vitamins in pressurised sprouted alfalfa seed stored in a refrigerator as compared with non-pressurised control.

Table 2. Losses (%) of vitamins in pressurised sprouted alfalfa seed during storage

Storage (days)	Vitamin C	Niacin	Pantothenic acid
1	77	11	7
3	83	61	60
7	92	60	60
14	94	59	59
21	92	61	62

With vitamin C, significant losses occurred during pressurisation (77% drop) and storage (additional 10–20% decrease). As can be seen, the contents of niacin and pantothenic acid were found declined by some 60% during the first three days of storage, with this level having been roughly maintained throughout the whole remaining storage time. The losses of niacin and pantothenic acid occurring during alfalfa pressurisation were 11% and 7%, respectively, which is very close to the values found recently (Mašková *et al*. 2000) for the decrease of vitamin B group in pressurised meat.

Sensory evaluation of sprouted, high pressure treated alfalfa seed

Figure 1 summarises the results of the sensory evaluation of pressurised and non-pressurised sprouted alfalfa seed. Sensory descriptors of germinated pressurised alfalfa seed were rated as inferior to those of non-pressurised control. Pressurisation exhibited the smallest influence upon flavour whereas the changes of texture and appearance were more significant.

All the sensory descriptors except for texture show a mild quality impairment in the course of storage (Figure 2) up to day 14. On day 21 of storage, all sensory descriptors indicate a marked deterioration. It follows from these results that, to avoid any

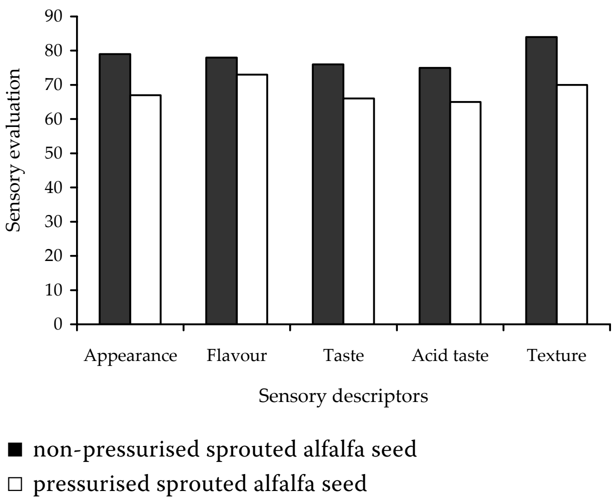

Figure 1. Influence of high pressure treatment on sensory quality of sprouted alfalfa seed (appearance, flavour, taste, acid taste, texture; 0 – unpleasant, 100 – very pleasant)

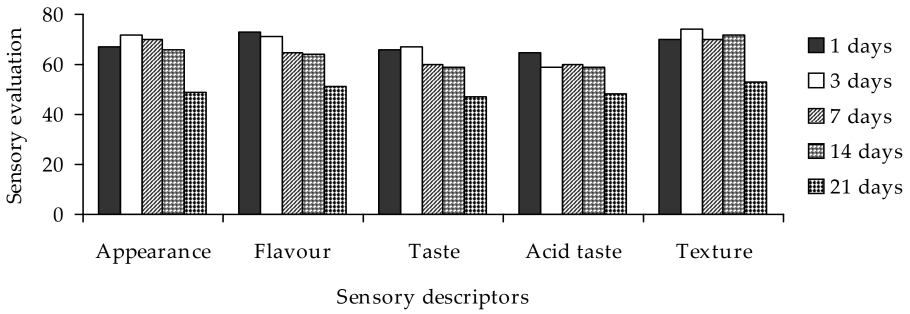

Figure 2. Changes in sensory descriptors of pressurised sprouted alfalfa seed during 21 days of storage (appearance, flavour, taste, acid taste, texture; 0 – unpleasant, 100 – very pleasant)

significant impairment of the sensory parameters of pressurised sprouted alfalfa seed, this should not be stored over 14-day period. However, even after 21 days of storage, the samples were recognised as acceptable, usable mainly in mixed salads. The influence of pressurisation on sprouted alfalfa seed itself is shown in the photographs (Figures 3 and 4).

To conclude the comments on the sensory evaluation, it can be stated that high hydrostatic pressure damaged the tissues of sprouted alfalfa seed which found an expression in an impaired appearance and texture. The damaged tissues also significantly influenced the sensory descriptors of sprouted seed during storage. After 21 days of storage, an

Figure 3. Sprouted alfalfa seed not treated by high hydrostatic pressure

Figure 4. Sprouted alfalfa seed treated with high hydrostatic pressure

Table 3. Microbiological evaluation of sprouted, high pressure treated alfalfa seed (in No./g)

Sample description	TMC ČSN ISO 4833	*Escherichia coli* ČSN ISO 16649-2	Yeasts ČSN ISO 7954	Coliform bacteria ČSN ISO 4832	Moulds ČSN ISO 7954
Without pickle, pressure 0	8.8×10^7	$< 1 \times 10^1$	$< 1 \times 10^1$	5.4×10^6	2×10^2
Pickled, pressure 0	1.5×10^4	$< 1 \times 10^1$	3×10^1	6×10^3	7.1×10^2
Pickled, 500 MPa, 10 min					
Stored days 0	3.2×10^2	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
1	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
7	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
14	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$
21	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$	$< 1 \times 10^1$

overall degradation of appearance, flavour, taste, acid taste, and texture became evident.

Microbiological evaluation of sprouted, high pressure treated alfalfa seed

Table 3 summarises the results of microbiological analyses. After the addition of the pickle, the total microorganism counts dropped from the original value 8.8×10^7 to 1.5×10^4, i.e. by more than three orders of magnitude; coliform bacteria counts also decreased by three orders of magnitude. High pressure treatment of the samples caused an additional decrease of microorganism counts (moulds by 1 order of magnitude, TMC and coliform bacteria by 2 orders of magnitude). No quantitative growth of microorganisms examined was observed during 21 days of storage and the samples showed full microbiological safety for the whole time.

CONCLUSION

Pressurisation significantly decreases microbial counts in sprouted alfalfa seed, thus prolonging its storage life from a few days to three weeks. The starting pickle acidity of pH = 2 keeps the pickle safely below pH = 4 all the time during pressurisation and storage. Pressurisation also influences the appearance and crispness of sprouted alfalfa. These impaired parameters represent no hindrance in utilising it in salads.

During pressurisation and storage, the content of vitamin C drops rapidly to 6–8% of the original value. The content of riboflavin is stable but insignificant. The contents of niacin and pantothenic acid decrease by some 60% during the first three days of storage and then do not change in subsequent days. From the viewpoint of nutrition, pressurised and subsequently stored sprouted alfalfa seed has little importance as a source of riboflavin, vitamin C, niacin, and pantothenic acid.

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