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FUNCTIONAL FOODS / FLAXSEED PROTEIN

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Abstract: *A field experiment which involved eleven flax varieties was conducted at the experimental site of the Agricultural Institute of Republic Srpska (abbr. The Institute) in Banja Luka in 2013. The continuation of the field research in 2014 was disabled by unprecedented flood. The first objective of the work was to determine the flaxseed yield and chemical composition. Relativelly low flaxseed yields on fertile alluvial soil were a consequence of summer drougt. Extremely high July's temperatures caused partial flaxseed rancidity before the harvest. Unlike flaxseed oil, the local consumers of flaxseed products have not been familiar with dietary benefits of flaxseed protein. For that reason, the second aim of this study was to provide basic information of the dietary functions of flaxseed protein and its components (functional food). This protein has similar pattern to soybean protein and in some respects even better characteristics. So, flaxseed protein has better lysine/arginine ratio. Both proteins have high Fisher's ratio (the ratio between essential branched and aromatic amino acids). Functional amino acid compounds with high Fischer's ratio show healing effects in the therapy of serious liver diseases. Though soybean provides higher protein yield than flax, the final income is higher with flaxseed due to very high price of flaxseed oil. Some mountain regions (Petrovačko polje) have excellent conditions for organic flax production. However, the organization and certification of organic production is feasible only with the support of governmental institutions. "Unpacking" of flaxseed on more functional food components or products (oil, protein, dietary fiber) presents a new challange for local researchers. Future activities should be focused on finding the cleanest technological process for the isolation of flaxseed protein.*

Key words: *flaxseed protein, functional foods, Fischer's ratio, organic production*

Introduction

Numerous research activities on flax as well as commercial flaxseed production (syn. linseed) in Bosnia and Herzegovina were launched during and after the Third Global Workshop "Natural Fibers for Healthy Life" held in Banja Luka in 2004. The organizers were the Agricultural Institute Banja Luka and the Institute for Natural Fibers from Poznan in the role of FAO European Cooperative Research Network on Flax and other Bast Plants. As all local flax varieties had been lost, the

first activities were focused on the introduction of foreign varieties of flaxseed (Garić and Mandić, 2004). Some mountain regions as Petrovačko polje were recognized to be suitable for "ecologically friendly" and organic flaxseed production (Nožinić, 2009; Nožinić et al., 2012; Nožinić et al., 2013). The ecological advantages of this mountain region are due to absence of weeds as *Ambrosia artemisiifolia* and unpolluted soils (some plots have not been cultivated for 30 years). Moderate summer temperatures with high number of sunny hours create almost ideal conditions in the period of flaxseed ripening. The production of cold - pressed vegetable oils at the Institute began in 2011. The transfer of knowledge from the Institute to the farmers resulted in about 30 oil mills which contribute higher farms' incomes. Having in mind that all components in the flaxseed show functional food effects, it would be a pity to "stay" on the linseed cake as a by - product. Covid pandemics and its consequences have turned the consumers' attention to the importance of functional foods. As the Institute's linseed oil production has been well elaborated, the next objective is to develop the production of sophisticated products like flaxseed protein, which is a source of valuable amino acids. More flaxseed products on the local farms (cold extracted oil, protein, fibers, mucilage, phytoestrogens) would insure higher incoms. If flax and flaxseed products were managed in an organic way, it would be a "winning combination". The flax varietal trials had been planned in 2013 and 2014. However, the flood in April and May 2014 strangled oilseed flax on the experimental field. For this reason, this paper deals with the results from one year (2013).

The objective of the research on flax varietal trial was to determine the variations among the varieties due to seed yield as well as chemical components. The second aim was to promote the importance of flaxseed protein in human diets.

Materials and methods

The experiment was conducted in the Institute's experimental field in Banja Luka in 2013 and 2014. The soil sample was taken two weeks before sowing and analysed on basic traits of fertility. The material consisted of eleven varieties (Table 1). The varieties (1 - 6) belong to oil form, while the varieties (7 - 11) belong to the fiber form. Flax was sown on April 17, 2013. The distance between rows was 25 cm, while the seed rate was 1.000 viable seeds/m^2. Mineral fertilizer, NPK 15-15-15 was applied before sowing in the amount of 300 kg/ha. Broad leaved weeds were controlled by the herbicide Basagran 480 (a.m. 480 g/lBentazon). Assessment of plant diseases were done in May and June. Harvest was done on July 29, 2013. Meteorological data from the closest station are presented in the Table 1 The chemical composition of the samples was done in the Institute's Laboratory of agroecology four months after the harvest. Protein content was determined by BAS EN ISO 5983 - 2: 2010, oil content by ISO ISO 6492:2008, and cellulose content by FOSS Ap.3801; AACC 32-10. Statistical analyse included calculation of mean values, interval of variation and coefficient of variation.

Agro ecological conditions in 2013

Table. 1. Climatic elements at the meterological station Banja Luka in 2013
Tabela. 1. Klimatski elementi za meteorološku stanicu Banja Luka u 2013. godini

2013	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Mean
Temperature	2.8	2.3	6.1	13.4	16.6	20.4	23.0	23.5	16.7	13.1	7.4	2.5	12.3
Max. temp.	16.9	18.6	20.7	31.8	31.7	36.0	41.6	41.1	30.2	29.0	25.0	17.7	41.6
Rainfall	94	116	89	63	120	54	27	36	70	68	156	0	893
Sunny hours	45	29	100	212	202	245	323	263	192	140	51	95	1896
Foggy days	12	8	5	1	2	1	0	0	1	5	13	15	63

Table. 2. Results of analyses of fertility at Banja Luka experimental site
Tabela. 2. Rezultati analize plodnosti na eksperimentalnom polju u Banja Luci

Depth	pH in H_2O	pH in KCl	Humus (%)	P_2O_5 mg 100 g	K_2O mg 100 g
0-25 cm	7.2	6.61	2.0	16.7	20.0
25-50 cm	7.1	6.60	1.6	14.3	17.1

Results and discussion

All ingredients of flaxseed (oil, protein, dietary fiber, phytoestrogens) offer functional food effects (Kajla et al., 2015). The technology of flaxseed oil production in the Institute's oil mill has been developing since 2011. Today's intention is to develop clean techniques for the extraction of flaxseed protein using available local resources and equipment. It is in accordance with the strategic approach due to ensuring sufficient quantity and appropriate quality of protein as a "weak point" in human and animal nutrition. Though soya is considered as a main protein crop, other crops like flax can contribute protein supply, especially from the aspect of quality.

Let us coment flaxseed yield and flaxseed protein yield in 2013. Though the alluvial soil at the experimental plot had excellent fertility (Table 2), the drought in June (Table 1) decreased flaxseed yield as well as flaxseed protein yield (Table 3). Flaxseed protein yield varied more than oil yield (Table 3). Two months later, soya on the neighboring plot with similar fertility provided the yield of 3000 kg/ha with 35% of proteine (1.050 kg proteine per ha). Though soya had much higher protein yield per hectar than flax (Table 3), the faxseed oil had a decisive role in the total profit per hectare. The price of flaxseed oil was even twenty times higher than the price of soya oil providing much higher total income per hectare than soya. The local market is not sufficiently informed of the benefits of soya oil (lecithin) for human consumption, as well as the benefits of flaxseed protein.

Table. 3. Results of flaxseed varietal trial in Banja Luka in 2013
Tabela. 3. Rezultati iz sortnog ogleda sa lanom u Banja Luci u 2013. godini

No.	Variety	Seed yield (kg/ha^{-1})	Oil (%)	Proteins (%)	Celulose (%)	Protein yield (kg/ha^{-1})	Oil yield (kg/ha^{-1})
1.	Local cultivar	1220	41.6	22.2	11.8	271	508
2.	Niagara	1455	41.4	23.9	10.0	348	602
3.	Flanders	1400	41.0	21.4	12.7	300	574
4.	Eole prelude oleagineux	1514	40.2	23.3	10.6	353	609
5.	Atalanta	1415	39.0	25.3	12.1	358	552
6.	Altess	1625	38.7	24.3	13.4	395	629
7.	Venica	1232	37.7	27.7	9.3	335	464
8.	Viking	1255	37.5	24.9	11.5	312	471
9.	Viola	1023	37.4	29.5	11.1	302	383
10.	Electra	1114	36.8	27.9	6.3	311	410
11.	Agatha	995	36.0	28.1	11.6	280	358
Mean		1295	38.9	25.3	10.9	324	504
Interval of variation		630	5.1	7.9	7.1	124	246
Coefficient of var.		15.7%	5%	10.4%	17.7%	11.5%	18.9%

Unlike flax production, succesfull soya production demands complex weed control by more herbicides as well the treatments with acarides and insecticides. Thanks to the environmental advantages in some mountain regions like Petrovačko polje, flax can be grown in the organic crop rotation systems (Nožinić et al., 2012). Due to the absence of aggressive weed *Ambrosia artemisiifolia* over 500 m of altitude, herbicides need not be applied. Virgin mountain soils, which have not been cultivated for years, offer satisfactory fertility for the flax production without mineral fertilizers. As flax must not be returned on the previous plot for a few years, additional advantage is regarding extensive agricultural production the possibility to change production locations for the flax production over large mountain valleys.

The shorter the path from flaxseed production in the field to the final products (oil, protein, dietary fibers), the greater the possibility of full control of their quality. It is due to the fact that flaxseed and flax products incline to spoilage processes during summer heat or in inadequate storage. Extremely high temperatures and strong light can lead to partial oxidation of omega three fatty acids in flaxseed in the field (before harvest). July's temperatures which reached 41.6˚C (Table 1) and late harvest caused a slight rancid taste of some flaxseed samples from the trial. However some farmers in the lowland region who harvested flaxseed in August 2013 faced a serious problem of flaxseed rancidity. Because of moderate summer temperatures in the mountain

regions, this problem has not been observed there. Since fatty acids are associated with some proteins (lipoproteins), oil rancidity reduces the value of flaxseed protein too. As the problem of rancidity can appear in the field or in the storage, the production and processing of flaxseed on the same place (farm) have evident advantages.

Table 4. Amino-acid contento f flaxseed, flaxseed flour and soy flour (g/100 g protein)
Tabela 4. Sadržaj amino kiselina u proteinu lanene sjemenke, lanenom i sojinom brašnu

Amino-acid	Brown linseed (NorLin)	Yellow linseed (Omega)	**Linseed flour	***Soy flour
Glutamic acid	19.6	19.7	22.8	18.6
Aspartic acid	9.3	9.7	8.3	11.7
Arginine	9.2	9.4	10.4	7.3
Leucine*	5.8	5.9	6.5	7.7
Glycine	5.8	5.8	4.9	4.0
Valine*	4.6	4.7	4.9	5.2
Phenylalanine*	4.6	4.7	6.5	5.1
Serine	4.5	4.6	4.1	4.9
Alanine	4.4	4.5	4.3	4.1
Isoleucine*	4.0	4.0	4.6	4.7
Lysine*	4.0	3.9	6.0	5.8
Treonine*	3.6	3.7	3.1	3.6
Proline	3.5	3.5	3.0	5.2
Tyrosine	2.3	2.3	4.6	3.4
Histidine*	2.2	2.3	5.9	2.5
Methionine*	1.5	1.4	3.0	1.2
Cystine	1.1	1.1	Not reported	1.1
Tryptophan*	1.8	-	-	-

Esential amino-acid*
Dev et al., 1986**
Friedman and Levin, 1989***

A series of lectures on the flaxseed products held in Banja Luka provided valuable information on the level of consumers' knowledge about these topics. It can be concluded that half of them were basically informed of flaxseed oil benefits, a few heard of mucilage role while no one was familiar with flaxseed protein components and functions. It is a reason to enlighten the role of flaxseed protein to potential consumers and patients with special dietary needs.

Cotyledons are the major oil and protein storage tissues. Main protein fractions are globulin (26-58 %) and albumin (20-42 %), (Dev and Sienkiewicz, 1987). By part, proteins are linked with the lipids (lipoproteins) and other molecules. For this reason, the separation of protein demands degrassing and dehulling of seed. Depending on processing technology, certain percentage of oil and dietary fibers remains in the protein product.

Flaxseed protein has a similar amino acid proffile like soybean protein (Oomah and Mazza, 1993). In some aspects, flaxseed protein has advantages over soy protein. The ratio of lysine/arginine in flaxseed protein flour (0.58) indicates that flaxseed protein is less lipidemic and atherogenic than soy protein meal (0.79) and other vegetable

proteins (Czarnecki and Kritchevsky, 1992), (Table 4). This ratio is higher in animal proteins than in plant proteins.

Flaxseed and soybean protein (specially some individual protein fractions) have high content of branched essential amino acids BCAA (Val + Leu + Isoleu) and low content of aromatic amino acids AA (Phe + Tyr), (Table 4). The higher the ratio between BCAA and AA (Fischer's ratio), the better effects in the terapy of liver failure, malnutrition associated with cancers, burns, trauma, acute diarrhoea and milk protein allergies (Weisdorf, 1998). Fischer's ratio significantlly varied among flaxseed protein fractions. The smallest rate (1.6) was determined in albumins and the highest (4.2) in the low molecular protein veight (Youle and Anthony Huang, 1981; Madhusudhan and Singh, 1985). Dietary intake of the functional food components with high Fischer's ratio (over 4.5) provides healing effects in the terapy of liver diseases (Kinny-Köster et al., 2016). These authors proved that mortality in patients with end-stage liver disease can be predicted by plasma amino acid concentrations.

Flaxseed protein is rich in arginine, aspartic acid and glutamic acid (Oomah, 2001).These amino acids are known to have strong effects on the immune functions of human and animal body. Arginine is a nutritionally essential amino acid for spermatogenesis and can be utilized as a potential agent to improve reproductive performance of boars under high ambient temperature. Results show that dietary arginine remarkably improved sperm motility, normality, total sperm number and effective total sperm number (Chen et. al, 2018). Because L-arginine acts as a vasodilator, opening (dilating) blood vessels, many people take oral L-arginine to treat hypertension, erectile dysfunction, preeclampsia and peripheral arterial disease.

Flaxseed protein products are used like stabilizers and emulsifiers in ice cream, sauces and meat emulsions (Martinez-Flores et al., 2006). This protein can be added in the gluten free foodstuffs in order to improve their quality (Gambus et al., 2009). Flaxseed proteins exhibit antifungal properties against *Alternaria solani, Candida albicans and Aspergillus flavus* (Xu et al., 2008). For this reason, they can be used as a natural preservative in the foodstuffs.

As the isolation of pure flaxseed protein is a very demanding process, the first Institute's protein products might contain a certain percentage of oil and dietary fiber. Although all ingredients of flaxseed have a positive effect on health, a higher admixture of oil in the protein product could accelerate its spoilage.

Conclusion

Production of functional foods, if possible in organic way, represents a great opportunity and challenge for domestic farmers and market. Each new functional food ingredient (in this case, flaxseed protein) provides higher incomes and better producers' motivation. The role of the government is to provide subsidies for producers of functional foods, and to support ongoing research programs in this field.

Future research activities on functional foods require a multidisciplinary approach including the best experts from the fields of agriculture, technology and medicine.

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FUNKCIONALNA HRANA / LANENI PROTEIN

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Sažetak: *Na oglednom polju Poljoprivrednog instituta Republike Srpske (skraćeno Institut) u Banjoj Luci 2013. godine sprovedeno je poljsko istraživanje koje je uključivalo jedanaest sorti lana. Nastavak istraživanja u 2014. godini onemogućila je velika poplava. Prvi cilj istraživanja odnosio se na utvrđivanje prinosa sjemena i hemijski sastav lanenog sjemena. Relativno mali prinosi sjemena posljedica su suše. Ekstremno visoke julske temperature izazvale su djelimično kvarenje sjemena prije žetve. Za razliku od lanenog ulja, lokalni potrošači lanenih proizvoda nisu bili upoznati sa prehrambenim prednostima lanenog proteina. Drugi cilj studije odnosi se na upoznavanje potrošača sa ulogom lanenog proteina i njegovih komponenti u ishrani. Ovaj protein ima sličan sastav i sadržaj amino kiselina kao protein soje, a u nekim aspektima čak i bolje karakteristike. Tako laneni protein ima bolji odnos između lizina i arginina. Oba proteina imaju visok Fišerov broj (odnos između esencijalnih lančastih masnih kiselina i aromatičnih amino kiselina). Funkcionalna jedinjenja amino kiselina sa visokim Fišerovim brojem pokazuju korisne efekte u terapiji teških oboljenja jetre. Iako soja daje veći prinos proteina od lana, konačni prihod je veći kod lanenog sjemena zbog veoma visoke cijene lanenog ulja. Neka planinska područja (posebno područje Petrovačkog polja) imaju odlične preduslove za organsku proizvodnju lana i lanenih proizvoda. Organizacija i certifikacija organske proizvodnje provodiva je samo uz podršku vladinih institucija. "Raspakivanje" lanenog sjemena na više funkcionalnih proizvoda predstavlja novi izazov za domaće istraživače. Buduće aktivnosti trebalo bi usmjeriti na iznalaženje što čistije tehnologije za izdvajanje lanenog proteina.*

Ključne riječi: *laneni protein, funkcionalna hrana, Fišerov broj, organska proizvodnja*