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MEDICINAL PROPERTIES AND MAIN INDICATORS OF SEED AND OIL QUALITY OF FLAXSEED - *Linum usitatissimum* L.

SUMMARY

Linum usitatissimum L. contains up to 45% oil. Linseed oil is a favourable fatty acid composition with a high linolenic acid content. Flaxseed oil is a valuable raw material for food and medical purposes due to its fatty acid composition and high content of linolenic acid. Omega-3 polyunsaturated fatty acids (PUFA) have shown that these compounds have therapeutic potential in several indications in neurology, psychiatry and cardiovascular disease. The aim of this study was to assess the quality of flax seed oils extracted from flax seed produced under different environmental conditions. Flax seed quality is highly dependent on weather conditions in the year of flax production, therefore the influence of the extreme weather events to the seed quality traits were also examined. The material consisted of nine samples of cold extracted oil from three flax varieties. Flaxseed oil production took place at five locations from 100 m to 700 m above sea level. The results indicate that high quality flax seed oil production is conditioned with proper farm technology, weather conditions and adequate storage. Oil rancidity and self - ignition of seed appeared in a case of improper seed storage. The expansion of the oilseed flax production should be accompanied by the education of farmers and potential consumers of flax products.

Keywords: *Linum usitatissimum* L., flaxseed oil, seed, sensory traits, chemical traits, medicinal properties

INTRODUCTION

Linum usitatissimum L. contains up to 45% oil. he fatty acid composition, especially the high linolenic acid content of linseed oil makes it a valuable raw material for food and medicinal purposes. Omega-3 polyunsaturated fatty acids (PUFA) have shown that these compounds have therapeutic potential in several indications in neurology, psychiatry and cardiovascular disease. Fiber flax had

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been a common crop throughout the Balkans until the introduction of man-made fiber. In some regions, local flax varieties were used for oil production too. Flaxseed contains 26–45% oil (Diedrichsen, 2001; Popović *et al.*, 2017, 2019a; 2021; Ikanović *et al.*, 2020; Ikanović and Popović, 2020). Approximately 22% of the oil is located in the seed coat and 4% in the embryo. The oil ispresent mainly as triacylglycerols in oil bodies having an average diameter of 1.3 μm (Daun *et al.*, 2003). Approximately 70% of all the linseed oil produced worldwide is destined for technical applications and 30% is for food production (Järvenpää, 2000). Flaxseed oil is used in a wide variety of applications, including additives in PVC plastics, antirust agents, laquers and paints, aroma substances for the food industry (Bonnarme *et al.*, 1997) or volatile com-pounds for obtaining a fresh green odour to off setthe decreased odour caused by the processing of vegetables (Noordermeer *et al.*, 2002). The lack of interest in this plant species had lasted until the end of the past century, so most of local varieties were irretrievably lost. The international conference "Bast Fibrous Plants Today and Tomorrow" held in St. Petersburg (September 1998) was a key event of importance for the return of flax to the Balkans, this time oil seed varieties (Kondić and Nožinić, 1998; Filipović *et al.*, 2013; 2021). In the following years, flax varieties from the gene bank "N.I. Vavilov" have been tested in the Institute's experimental field in Banja Luka. Commercial oil seed flax production (syn. linseed) has been developing after the Third Global Work shop of the FAO/ESCORENA European Cooperative Research Network on Flax and Other Bast Plants "*Bast Fibrous Plants for Healthy Life*" held in Banja Luka in 2004. The Institute for Natural Fibers from Poznan (director prof. Richard Kozlowski) and the Institute in Banja Luka organized this event in very sucesfull way. As local flax varieties had been lost, the first activities were focused on the introduction of flax seed from the Agricultural Institute in Zajačar in Serbia (Garić and Mandić, 2004). Some mountain regions (Petrovačko polje, Manjača) were recognized to be suitable for "ecologically friendly" and organic oil seed flax production (Nožinić, 2009; Nožinić *et al.*, 2012; 2013; 2016). The ecological advantages of mountain regions are due to the absence of invasive weeds as *Ambrosia artemisiifolia* and unpolluted soils (some plots have not been cultivated for 30 years). The production of cold extracted 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 have been contributed to farms' incomes. Flax seed, flax seed ingredients, especially flax seed oil show evident functional food effects what has been proved in many research studies (Oomah and Mazza, 1998; Oomah, 2001; Mani *et al.*, 2011; Rodriguez *et al.*, 2013; Caligiuri *et al.*, 2014; Glamočlija *et al.*, 2015; Caligiuri *et al.*, 2016; Parikh *et al.*, 2018). For this reason flax seed products reach high price on the local market. However, as flax has a shallow root system, its production in the lowlands is more and more difficult for frequent droughts and global warming. Extremely high temperatures can affect seed and oil quality even before harvesting. Unlike lowlands, some mountain valleys with moderate summer temperatures, high number of sunny hours and permanent wind

activity create almost ideal conditions in the period of oil seed flax ripening. Flax requires certain storage conditions. Banal mistakes, such as storing seeds in a thick layer in a plastic package can lead to product spoilage or even self-ignition. So, it is not easy to produce quality flax seed oil and earn desired money. The aim of this paper was to describe the quality of flax seed oil samples extracted from flax seed produced in different agroecological conditions.

MATERIAL AND METHODS

Analysed material consisted of nine samples of cold extracted oil from three flax seed varieties. The first oil sample (BL-Z) was extracted from yellow seed variety Zlatko (eng. "Golden") produced at the Institute's experimental field in Banja Luka. The second oil sample (BL-O) was extracted from Romanian variety Olin (brown flax seed) harvested on the same location. Other oil samples (Table 1) originate from one local flax variety. Its origin and classification (oil or fibre form) are not clear. This seed had been stored on the old house roof in the village Vrtoče for many years, then sown in 2004. Since 2004, the seed has been multiplied on more locations with different climate conditions showing excellent adaptability. The oil sample (OR) originated from Orašje, a fertile lowland area besides the River Sava (Table 1). The organoleptic characteristics of flax seed and flax seed oil were evaluated on the basis of the visual observations, odor and taste. The flaxseed diseases were determined in the Laboratory for phytopathology at the Agricultural institute of Republic Srpska (abbr. Institute). The oil was extracted in the Institute's oil mill. Chemical analysis of oil included Peroxide value (PV), free fatty acids (FFA) and content of fatty acids. As flax seed quality is highly dependent on weather conditions in the year of flax production, the influence of the extreme weather events to the seed quality traits were commented too.

Table 1. Basic data of flaxseed production locations

Location	Longitude	Latitude	Altitude (m)	Relief form
Banja Luka	17.2200	44.7800	About 150	River valley
Vedro polje	16.3896	44.5064	650 - 700	Mountain valley without river
Vrtoče	16.1753	44.6347	About 600	Mountain valley without river
Derventa	17.9067	44.9792	150 - 200	Gentle slopes
Orašje	18.6935	45.0368	About 100	Large river valley

Other oil samples were extracted from flaxseed produced in the region of the Petrovačko polje (abbr. PE). The abbreviation (PE-VP) is related to the seed produced in the valley Vedro Polje (eng. "Clear Field"), which is a part of Petrovačko polje (PE). The name of this valley corresponds to its main climatic feature (high number of sunny hours). The abbreviation PE-V is related with the village Vrtoče in the central part of Petrovačko Polje. The spreading of local variety began by the enthusiast Miodrag Latinović from that village. The

organoleptic flax seed properties were evaluated on the basis of the visual flax seed characteristics (smooth or wrinkled seed surface, seed gloss, seed size, visible traces of pathogens or pathogen activity on seeds), odor and taste. The diseases were determined in the Institute's Laboratory for phytopathology at the Agricultural institute of Republic Srpska (abbr. Institute). The oil was extracted in the Institute's oil mill. Chemical analyses of oil included Peroxide value (PV), free fatty acids (FFA) and content of fatty acids, have been done by the Laboratory of "Bimal Group" in Brčko. Peroxide value (PV) was determined by method ISO 3960, free fatty acids (FFA) were determined according to ISO 660 and content of fatty acids by GC methodology (ISO 5508, 5509). The influence of the extreme weather events to the seed quality traits was described on the basis of climatic data from the meteorological stations Banja Luka and Drinić - Petrovac.

The result of soil sample from Vedro Polje is quite representative for other locations on Petrovačko Polje (Table 2). These soils are shallow and the depth depends on the position in the mountain field. The content of humus and potassium is high while available phosphors is in deficit. Thanks to dolomite stone under the soil, its reaction is neutral. The alluvial soil in the valley of the river Vrbas (experimental field of the Agricultural Institute has relative good fertility while physical traits are upset with intensive irrigation of previous crop (hybrid maize). The alluvial soil in Orašje belongs to the category of deep alluvial soils with high fertility.

Table 2. Soil fertility traits at flax plots

Location/trait	Year	Depth in cm	pH in H_2O	pH in KCl	Humus (%)	P_2O_5 mg/ 100 g	K_2O mg/ 100 g
Banja Luka	2009	0-25	7.2	6.6	3.1	16.7	23.0
Vedro polje	2014	0-25	7.5	6.7	7.0	2.0	47.0
Derventa	2013	0-25	5.8	5.3	2.1	1.1	13.4
Orašje	2013	0-25	6.4	5.8	4.3	13.8	20.0

Table 3. Total precipitation (P, lit./m^2) and maximum temperatures (T, ˚C) in Banja Luka (150 m alt.) and Drinić (730 m alt.) in the flax vegetation seasons

Year/month	April		May		June		July		August	
	P	T	P	T	P	T	P	T	P	T
BL, 2009	40	26.4	49	34.1	153	35.7	43	38.1	138	37.4
BL, 2013	63	31.8	120	31.7	54	36.0	27	41.6	36	41.1
BL, 2014	214	25.8	217	31.2	97	34.1	139	34.3	177	34.1
DR, 2014	221	22.0	217	29.4	157	31.8	174	32.4	177	32.6
BL, 2018	20	31.0	137	31.1	103	34.5	84	34.0	82	35.3
DR, 2018	16	25.3	122	26.5	81	29.0	28	29.2	106	31.5
BL, 2019	105	29.9	225	27.2	123	35.3	59	36.8	49	37.7
BL, 2020	27	28.5	104	30.0	62	35.1	72	35.7	142	36.7
BL, 2021	69	27.8	82	31.1	12	39.3	63	40.2	57	-

Abbrivitatons: BL - Banja Luka, DR - Drinić

The influence of the extreme weather events to the seed quality traits was described on the basis of climatic data from the meteorological stations Banja Luka (150 m alt.) and Drinić at the edge of Petrovačko polje (700 m alt.), Table 3. Though Derventa and Prnjavor do not have meteorological stations, the temperature regime from the station Banja Luka is quite representative for these production regions too. The meteorological data for the station Drinić have not been available for all vegetation seasons. Comparable data were available for 2014 and 2018.

RESULTS AND DISCUSSION

Flax seed production parameters

Proper agrotechnique from sowing till harvesting is the first precondition for high quality flaxseed. Because of the weak root system and specific ecological requirements in the ripening period, oilseed flax belongs to the crops which are very vulnerable to the extreme weather conditions (Popović *et al.*, 2017; 2021, 2022a; b). The quality of flax seed in 2013 was highly dependent on harvest date. Better seed quality was obtained when the harvest was done till the mid of July. The flax seed from Orašje (OR), which was harvested on July 12 provided oil with excellent taste as well as desirable PV and FFA percentage (Table 4, Figure 1). The flaxseed that was maturing in the second part of July and in the first part of August was exposed to extreme heat. In the third decade of July 2013, the lowland regions experienced maximum temperatures about 40˚C (Banja Luka, 41.6˚C).

Table 4. Results of flaxseed oil analyses, g*

Sample	Harvest	Analysed	PV	FFA	C18:3	C18:2	C18:1	C18:0	C16:0
BL-Z	2009	IV, 2010	0.31	0.69	56.0	15.2	18.0	3.6	5.2
BL-O	2009	IV, 2010	0.21	0.51	54.2	12.6	20.6	4.4	5.8
OR	2013	IX, 2013	1.74	0.21	50.3	12.4	25.5	4.4	6.1
PE-V	2014	III, 2015	2.99	0.29	61.7	14.7	15.3	3.6	4.5
PE-V	2019	XII,2019	0.00	0.36	59.6	12.9	17.6	3.5	5.5
PE-VP	2020	I, 2021	0.66	0.64	60.1	12.3	18.9	3.6	4.7
PE-VP	2019	I, 2021	0.34	0.43	-	-	-	-	-
PE-VP	2018	I, 2021	10.46	3.20					
DE	2020	III, 2021	15.03	4.11	53.2	12.9	18.1	3.5	4.9

*PV-Peroxide value; FFA-free fatty acids; C16:0-palmitic acid; C18:0-stearic acid; C18:1-oleic acid; C18:2-Linoleic acid; C18:3- alfa-linolenic acid, ALA

The year 2014 can rightly be called the "Black year" for oilseed flax production. Heavy rains in the period April - September (Banja Luka, 944 lit/m^2; Drinić - Petrovac, 1.246 lit/m^2) led to the development of serious flax diseases (flax wilt), which caused the decay of plants in the period of emergence as well as rapid reduction of flaxseed yield and quality. The unprecedented flood in April 2014 strangled the flax in the varietal trial at the Institute's experimental field in Banja Luka. Small quantity of flax seed harvested in 2014 had significantly lower germination in 2015. Flax survived stress conditions in 2014 just on a few plots

with permeable soils in the mountain region of Petrovačko polje. The oil from one sample of the flaxseed produced in 2014 was analysed in 2015. Although the content of PV and FFA indicated relative good oil quality (tab. 4). Almost all oil seed flax fields in 2014 were infested by *Fusarium oxysporum*, which is a common fungi in the humid conditions. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. lini (Fol), can infect flax at any growth stage and may result in 100% disease incidence in certain cultivars (Panjan, 1968; Kommedahl *et al.*, 1970). The pathogen which can be seed-borne or soil-borne, invades through roots and develops in the xylem. Radman (1978) states that this pathogen makes more damage on oilseed flax varieties than fiber ones. Very high content of linolenic fatty acid (omega - 3) in the oil from the seed harvested in 2014 might be the result of extremely long vegetation. The longer vegetation, the higher the content of polyunsaturated fatty acids in oil crops (Kastori, 1991).

Perfect flaxseed oil was produced from the flaxseed harvested in the village Vrtoče (PE-V) in 2019 (Table 4). Total absence of the reactive oxygen in this oil sample indicates proper production technology and proper oil storage. Thanks to proper field production technology and absence of invasive weeds (*Ambrosia sp.*), there is no need for herbicide treatment. It is an organic model of production, without certification. As all activities "from the field to the oil" take place under full farmer's control, all production risks are reduced to minimum.

Oil samples from “Vedro polje” (eng. Clear Field) had excellent quality except for 2018. The name of the valley indicates desirable environmental condition for flax seed oil production.

Flaxseed oil has the highest needs in water during the phases of the intensive growth and flowering in May and June. Since June 2021 was the driest one (Banja Luka 12 lit/m^2) in the period of measurements (Banja Luka, since 1881), shallow root system of flax could not provide enough water for normal flax growth and pollination. Moderately warm and sunny weather in July stimulates oil synthesis and ripening of flax seed. July 2021 brought hellish heat (Banja Luka, max. 40.2˚C), which caused shrinking of flaxseed and negligible yield. Evident is negative effects of extreme weather conditions to flax production.

Improper flaxseed storage can cause spoilage and great damage. The oil from improperly stored seed in region of Derventa (DE) had a bitter taste, too high PV and FFA percentage (Table 4). Rancid oil can be used for natural protection and decoration of woody surfaces.

Compared with the lowlands, mountain region in “Petrovačko polje”, he had more sunny hours and less foggy days. Rich mountain relief and closeness of the Adriatic Sea create permanent winds' activity. The more air circulation, the less plant diseases appeare. Unlike lowlands, the temperatures in the mountain valleys rarely exceed 30˚C. Such moderate temperatures favor longer oil synthesis providing oil with a very high content of polyunsaturated fatty acids (omega-3). The fact is that “Petrovačko polje” meets all conditions for the flax seed products with geographical origin.

Two oil samples (BL-Z, BL-O) from the seed harvested at the Institute's experimental field in Banja Luka in 2009 had excellent taste, low PV and FFA percentage, which indicated proper seed and oil management. That year was relatively suitable for oilseed flax production. Typical June with high rainfall (Banja Luka, 153 l/m^2) provided satisfactory conditions for normal growth, pollination and seed development. Though frequent rains caused occurence of new flowers for longer periods (partial retrovegetation), dry and hot weather in July favored uniform flaxseed ripening. The Serbian variety Zlatko was released in 2003 in the Agricultural Institute in Zaječar (Stanković *et al.*, 2003), then tested in Banja Luka region in the period 2006-2014. Unfortunately, this valuable variety was lost during the flood in 2014. Romanian variety Olin provided excellent oil yields in the mountain region of Manjača (village Sitnica). This oil had a very "soft" taste, acceptable for consumers.

Having in mind that domestic oilseed flax production is limited on one old variety, it is time for new genetics. New oilseed flax varieties, NS Primus and NS Marko, selected in the Institute of Field and Vegetable Crops in Novi Sad obtain extremely stable and good yields, have good adaptability and high nutritional value (Popović *et al*., 2017, 2021, 2022b).

Some medicinal effects of flaxseed ingredients

All ingredients of flaxseed (oil, protein, dietary fibers, phytoestrogens, mucilage, vitamins) offer health benefits, so can be used in medicinal purposes like functional foods or pharmaceutical products (Popović *et al.*, 2017, 2019a; 2019b; 2021; 2022). Flaxseed contains 26–45% oil (Diedrichsen, 2001; Popović *et al*., 2017, 2019a; 2021; Ikanović *et al*., 2020; Ikanović and Popović, 2020). The oil is present mainly in oil bodies having an average diameter of 1.3 μm (Daun *et al*., 2003). Flaxseed (syn. linseed) is one of the richest plant sources of the ω-3 fatty acid alpha-linolenic acid (ALA, C18:3 ω-3), vitamins of groups A, B and E and used in the food and pharmaceutical industry (Popović *et al.*, 2017, 2019a; 2019b; 2021; 2022b), Figure 1.

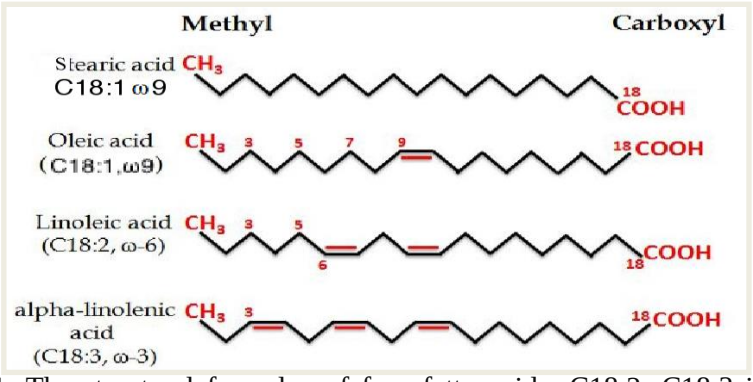

Figure 1. The structural formulae of four fatty acids. C18:2; C18:3 is dietary essential fatty acids (Popović *et al*., 2019a; 2022b)

Flaxseed oil is the richest herbal source of alpha-linolenic acid (ALA) what is of great importance for preventing the occurrence of cardiovascular disease (CVD) with nutritional interventions. The increased use of omega-3 fatty acids can be a powerful example of nutritional strategy that may produce significant cardiovascular benefits. Because of its high ALA content, the use of flax seed has been advocated to combat CVD (Rodriguez-Leyva *et al*., 2010).

The author states that sixty patients of type 2 diabetes were fed a daily diet for 3 months, with flax seed gum was incorporated in wheat flour chapattis, along with six wheat flour chapattis containing flax seed gum (5g), as per recommendations of the American Diabetic Association. Biochemical blood profiles in the control group, which consumed an identical diet but chapatis were without chewing gum, showed that fasting blood sugar in the experimental group decreased from 154 ± 8 mg/dl to 136 ± 7 mg/dl (P= 0.03) and total cholesterol from 182 ± 11 mg/dl to 163 ± 9 mg/dl (P=0.03). Results showed a decrease in low-density lipoprotein cholesterol from 110 ± 8 mg/dl to 92 ± 9 mg/dl (P=0.02) and the efficacy of flax gum in the blood biochemistry profiles of type 2 diabetes (Thakur *et al.*, 2009).

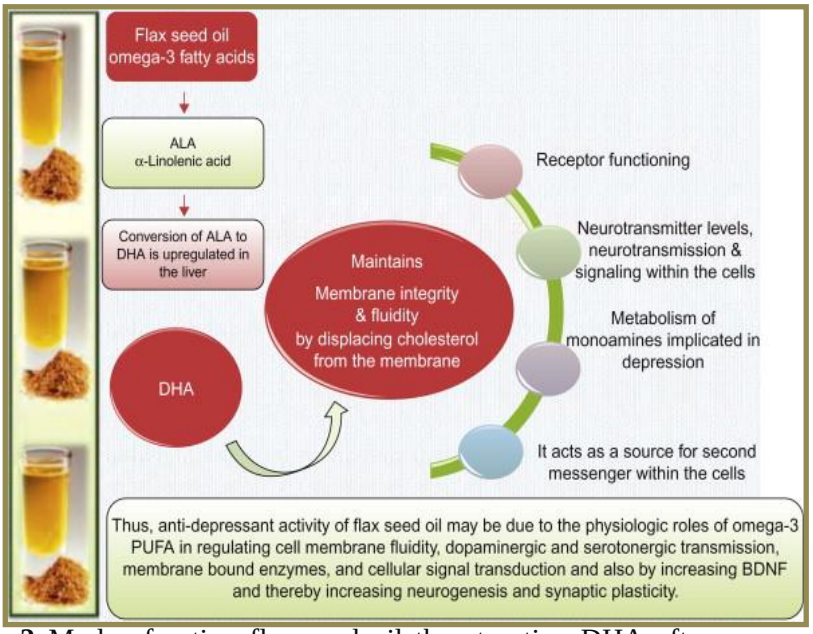

Figure 2. Mode of action flax seed oil throut action DHA after conversion of ALA and potential health effects (Chandola & Tanna, 2014; Popović *et al*., 2022b)

Reducing and preventing the occurrence of cardiovascular disease (CVD) through nutritional interventions is a therapeutic strategy with increased use of omega (ω)-3 fatty acids that can produce significant cardiovascular benefits. Flaxseed is one of the richest sources of the plant-based ω-3 fatty acid, alpha-

linolenic acid (ALA). Based on the results of clinical trials, epidemiological investigations and experimental studies, ingestion of ALA has been suggested to have a positive impact on CVD. Because of its high ALA content, the use of flax seed has been advocated to combat CVD (Chandola and Tanna, 2014; Calado *et al.*, 2018).

Flax seed is a rich sourse of ALA which is endogenously converted into DHA. DHA, by mantaining membrane fluicidi, by displacing holesterol from the membrane, corrects receptor functioning, regulates neurotransmiter levels, neurotransmision, and singnaling within the cells. Its acs as a second messenger sourse with cells (https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/linseed-oil), Figure 2. It helps in metabolism of monoamines implicated (Derman *et al.*, 2005; Watson, 2014) etiopathogenesis of depresium. Many studies with omega-3 polyunsaturated fatty acids (PUFA) indicate that these compounds have therapeutic potential in neurology and psychiatry. Omega-3 PUFA leads to increased survival of neurons and glia, and improved neurological outcome. The DHA injection led to an increased neuronal and glial cell survival, and the effect of the DHA injection was amplified by addition of DHA to the diet (Michael-Titus, 2007). Flaxseed is rich in omega-3 fatty acids, α-linolenic acid, lignan, and fibers. Flaxseed lignans, 95% are made of the predominant secoisolariciresinol diglucoside (SDG), which is converted into enterolactone and enterodiol, both structurally similar to estrogen, they can bind to cell receptors, decreasing cell growth (Figure 3). Results have shown mixed findings, and much more human research is needed. Intake of omega-3 fatty acids is related to the reduction of breast cancer risk, prostate and colon (Calado *et al.*, 2018; Popović *et al.*, 2019a; 2021).

Figure 3. Flaxseed oil and healt benefit; https://www.lybrate.com/topic/benefits-of-flax-seed-oil-and-its-side-effects; Popović *et al.*, 2022b.

Treatment with omega-3 PUFA could represent a promising therapeutic approach in the management of neurological injury. Bifidobacterially produced C18:3 CFA may have potential in the control of colon cancer (Hennessy *et al.*, 2016). The therapeutic potential in the gastrointestinal tract is indicated by their inhibitory properties against common gastrointestinal pathogens (especially methicillin-resistant *S. aureus* ATCC 43300).

CONCLUSION

Flax seed oil quality is highly dependent on proper technology production, locality and environmental conditions. Evident is negative effects of extreme weather conditions to flax production. Oil seed flax has the highest needs in water during the phases of the intensive growth and flowering in May and June. Mountain region in "Petrovačko polje" provides better agro ecological conditions for oil seed flax production than low lands. Rich mountain relief and closeness of the Adriatic Sea create permanent winds' activity. He had more sunny hours and less foggy days compared with the lowlands.Unlike lowlands, the temperatures in the mountain valleys rarely exceed 30˚C. Moderately warm and sunny weather in July stimulates oil synthesis and ripening of flax seed. Such moderate temperatures favor longer oil synthesis providing oil with a very high content of polyunsaturated fatty acids (omega-3). "Petrovačko polje" meets all conditions for the flax seed products with geographical origin.

All ingredients of flaxseed (oil, protein, dietary fibers, phytoestrogens, mucilage, vitamins) offer health benefits, so can be used in medicinal purposes like functional foods or pharmaceutical products. The increased use of omega-3 fatty acids can be a powerful example of nutritional strategy that have therapeutic potential in neurology and psychiatry, may produce significant cardiovascular benefits and cancer prevention.

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