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ORIGINAL PAPER

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FATTY ACID PROFILE, ATHEROGENIC AND THROMBOGENIC HEALTH LIPID INDICES OF LYOPHILIZED BUCKWHEAT SPROUTS MODIFIED WITH THE ADDITION OF *SACCHAROMYCES CEREVISIAE* VAR. *BOULARDII*

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ABSTRACT

Background. The study aims to present an assessment of the effect on the composition of fatty acids of a modification of buckwheat sprouts *Fagopyrum esculentum* Moench by the addition of the probiotic strain of yeast *Saccharomyces cerevisiae* var. *boulardii.* The study is innovative.

Materials and methods. Seeds, control and modified buckwheat sprouts lyophilisates constituted the research material. Fat analyses were performed using the standards methods. However, the determination of fatty acids was carried out following the AOCS Ce 2-66 methodology.

Results. The results indicated that the germination process increased the total fat content of the sprouts as well as changed the fatty acid profile. Statistically significant differences were found in the content of palmitic, arachidic, ginkgolic, oleic, eicosenoic and linoleic acids between the control and probiotic-rich sprouts. It was also found that the quality indicators of buckwheat lipids, such as atherogenic and thrombogenic, are optimal in terms of nutritional value.

Conclusions. Buckwheat sprouts modified by adding probiotic yeast might be a new functional product that can be used as part of a diet that reduces the risk of cardiovascular disease.

Keywords: *Fagopyrum esculentum* Moench, fatty acids, lipid indices, functional product

INTRODUCTION

Buckwheat belongs to the knotweed family, i.e. *Polygonaceae*, and is classified as a pseudocereal (Zhang et al., 2012). One of the most commonly grown species of buckwheat is *Fagopyrum esculentum* Moench. The global production of buckwheat in 2017 was 3,972,882 million tons, double the level in 2014 (1,915,966 million tonnes) (FAOSTAT, n.d.). This pseudocereal is a well-known source of health-promoting ingredients such as proteins with a high biological value, a well-balanced amino acid pattern and antioxidant

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compounds (rutin, quercetin, orientin, isoorientin, vitexin, isovitexin and vitamins). Germination has a positive effect on the biochemical changes and the nutritional value of germinated seeds (raw material). During this process, many changes occur that affect the increase or decrease in compounds contained in the raw material, including the use of compounds necessary for growth. Storage substances are broken down and also used for plant growth, which makes them easier to absorb. Fats, protein and starch are broken down into energy source compounds and substrates from which newly synthesized compounds are formed. This is essential information in the context of functional foods and industry that uses this raw material (Benincasa et al., 2019; Brajdes and Vizireanu, 2012; Lewicki, 2010; Zhang et al., 2012; Zhang et al., 2015).

Compounds contained in buckwheat exhibit a number of health-promoting properties and activities, including lowering plasma cholesterol (e.g. through buckwheat protein, flavonoids), neuroprotective effects (through tyrosinase, acetylcholinesterase, butyrylcholinesterase inhibitors, antioxidant activity; Đurendić-Brenesel et al., 2013; Gulpinar et al., 2012; Zhang et al., 2012; Zhou et al., 2015).

Probiotic microorganisms may exhibit pro-health activity through certain mechanisms of action, such as trophic effects, immune regulation and related antimicrobial properties. *Saccharomyces cerevisiae* var. *boulardii* has a wide medical application, related among other things to the prevention of antibiotic-related diarrhea and traveler's diarrhea; it also appears to be effective in preventing relapse in patients with Crohn's disease (Kelesidis and Pothoulakis, 2011; Lazo-Vélez et al., 2018; Łukaszewicz, 2012; Syngai et al., 2016). The publication by Ryan et al. (2015) indicated that supplementation with *S. boulardii* reduced the level of remnant lipoprotein, which is considered a potentially therapeutic factor in the treatment and prevention of coronary artery disease. One of the mechanisms that alters cholesterol levels is its assimilation to yeast cells (Ryan et al., 2015). It is very important to mention that this species of yeast is recommended as a biological agent that can be intentionally added to food or feed, according to the EFSA notification 8 (Ricci et al., 2018).

According to the WHO definition: "(...) when administered in adequate amounts, probiotics confer a health benefit on the host" and can be found in many products on the market, e.g. fermented dairy products, which are eliminated by people on a vegan diet (FAO and WHO, 2002; Pala et al., 2011; Tripathi and Giri, 2014). In turn, this creates a niche on the market for products that will be able to provide probiotic microorganisms to this group of consumers. In the study by Świeca et al., the possibility of using legume sprouts as carriers for probiotic bacteria was assessed and it was found that soybean sprouts were their best carrier (Świeca et al., 2017). In contrast, another study by the same author found that leguminous sprouts enriched in *Saccharomyces cerevisiae* var. *boulardii* are a new functional product, which is characterized by high health and nutritional properties (Świeca et al., 2019), hence the hypothesis about the combination of *Saccharomyces cerevisiae* var. *boulardii*, but with pseudocereal. This combination may change the composition and increase the nutraceutical effect of the raw material.

In connection with the above, the purpose of this work is to present the effect of modification of buckwheat sprouts *Fagopyrum esculentum* Moench by probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* to change the composition of fatty acids that increase the health potential of this raw material.

MATERIAL AND METHODS

Buckwheat seeds were purchased from the PNOS SA in Ożarów Mazowiecki, Poland.

Preparation of control and probiotic-rich sprouts

Strain of *Saccharomyces cerevisiae* var. *boulardii* (confirmed microscopically by sequencing the region containing the 3 'end of 18S rDNA, ITS1, 5.8S rDNA, ITS2 and the 5' end of 26S rDNA and by biochemical tests) was maintained on YPG agar slants (BTL, Łódź, Poland) at 4°C. It was cultured on malt agar for 48 hours at 30°C. Colonies of yeast were collected in a sterile manner from Petri dishes. They were suspended in water and then used to inoculate the seeds. The standard curve that was established was then used to determine the number of yeast cells *Saccharomyces cerevisiae* var. *boulardii* in a suspension at a level of 1×10^7 ml^{-1} based on the OD value. For preparation of the inoculum, the cell concentration was estimated by optical density (OD) analysis

at 600 nm using a spectrophotometer Smart Spec Plus (Bio-Rad, USA).

Seeds were disinfected in 1% (v/v) sodium hypochlorite for 10 min, then drained and washed with distilled water until they reached a neutral pH. Then, they were soaked in *Saccharomyces cerevisiae* var. *boulardii* water suspension (1×10^7 CFU per 1 gram of seeds; S, soaked with probiotics) or distilled water (C, control). Buckwheat seeds were imbibed for 4 hours. The seeds (approximately 12.5 g per plate) were dark-germinated for three days in a growth chamber (SANYO MLR-350H) on Petri dishes (φ 125 mm) lined with absorbent paper (relative humidity 90%). Seedlings were sprayed daily with Milli-Q water. Sprouting was run at 30°C. After three days, sprouts were manually collected and rinsed with distilled water. Microbiological tests were carried out immediately.

The material (grains, control and probiotic-rich sprouts) was frozen and lyophilized in Labconco Freezone 1L Freeze Dry System for nutritional and prohealth analysis.

Microbiological quality

The following microbiological analyses were performed in accordance with European or Polish standards.

Total mesophilic bacteria. The total number of mesophilic bacteria was determined with the plate technique on nutrient agar, according to PN-EN ISO 4833-2:2013 (Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 degrees C by the surface plating technique.

Yeasts and molds. Yeast and molds were differentiated according to the morphology of colonies. Their numbers were determined using the plate technique on glucose, yeast extract and chloramphenicol agar according to PN-ISO 21527-1:2009 (Microbiology of food and anima..., n.d.).

Determination of crude fats

The fat content of lyophilisates: seeds, controls and modified sprouts was determined using the Avanti Soxtec System (Model 2055 Manual Extraction Unit; Foss Tecator, Höganäs, Sweden).

Fat extraction

The fat from the freeze-dried seeds, control and probiotic-rich sprouts was extracted using the procedure described by Folch et al. (1957).

Analysis of fatty acid composition

GC-FID determined the percentage fatty acid composition. Fatty acid methyl esters were obtained according to the AOCS Official Method Ce 2-66 (n.d.).

Chromatographic separation was performed using Hewlett-Packard 5890 II apparatus equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm × 0.25 μm) operating at programmed temperature conditions: from 60°C, at 12°C/min to 200°C maintained for 25 min. The temperature of the injection chamber and the detector was 240°C. Fatty acids were identified by comparing retention times with standards (Supelco 37 Component FAME Mix), and the results were reported as weight percentages following integration and calculation using a ChemStation (Agilent Technologies). This analysis were carried out in triplicate.

Indexes of lipid quality

Based on the fatty acid composition data, the atherogenicity index (IA) and the thrombogenicity index (IT) were calculated. The following equations were applied:

$$IA = [C12{:}0 + 4 \times (C14{:}0) + C16{:}0] / (MUFA + PUFA) \quad (1)$$

$$IT = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times MUFA + 0.5\ (n\text{-}6) + 3 \times (n\text{-}3) + (n\text{-}3 / n\text{-}6)] \quad (2)$$

where PUFA stands for polyene fatty acids (in the IA equation it is the sum of *n*-6 and *n*-3 fatty acids) and MUFA stands for monoenoic fatty acids (Ulbricht and Southgate, 1991).

Other lipid indices expressing the ratio of polyene fatty acids to saturated fatty acids (PUFA / SFA), the sum of polyene and monoenoic fatty acids to saturated fatty acids (UFA / SFA), and the ratio of *n*-6 to *n*-3 (*n*-6 / *n*-3) were calculated (Sinkovič et al., 2020).

Statistical analysis

Statistical analysis of the data was performed using Statistica 10 (StatSoft, Tulsa, OK, USA). For statistical

analysis, one-way analysis of variance and intergroup differences was used by Tukey's HSD post-hoc test with a significance level of $P < 0.05$, $P < 0.01$ and $P < 0.001$. Significant differences were denoted with different superscript letters.

RESULTS AND DISCUSSION

Germination is a natural biological process that occurs in the seeds of pseudocereals such as *Fagopyrum esculentum* Moench, *Fagopyrum tataricum* (L.) Gaertn., *Amaranth caudatus* L. This process affects a number of changes in seeds, including fat degradation to free fatty acids (Guardianelli et al., 2019; Marton et al., 2010).

Microbiological analyses

The amount of TMB, yeast and mold in the controls and probiotic sprouts is shown in Table 1. It was proved that after 3 days of sprouting a single edible portion of sprouts enriched with probiotic yeast contained an amount classifying them as a probiotic product (6.5 log/100 g f.m.). Co-culture of *Saccharomyces cerevisiae* var. *boulardii* and buckwheat caused a slight increase in the total count of mesophilic bacteria. It may be suggested that probiotic yeast caused a loosening of seed structure, which promotes the growth of other microorganisms.

Table 1. Count of TMB, yeast and molds in the control and probiotic-rich sprouts

Buckwheat forms	Count of microorganisms log 10 CFU/g f.m.		
	total mesophilic bacteria	yeasts	molds
Control sprouts	$7.13^{a,***}$±0.02	$1.43^{a,***}$±0.15	$0^{a,***}$
Probiotic-rich sprouts	$7.99^{b,***}$ ±0.03	$4.53^{b,***}$±0.05	$0^{a,***}$

Mean values with different letters in the column are statistically significantly different (***, $P \leq 0.001$). "±" means standard deviation.

Total fat content

During the germination process, the total amount of fat increased in sprouts ($P < 0.001$). The fat content is shown in Figure 1. Compared to the dormant seeds the total fat content in the control sprouts and probiotic-rich sprouts was higher by c. 50%. There were no statistical differences between the control sprouts and those enriched with yeasts.

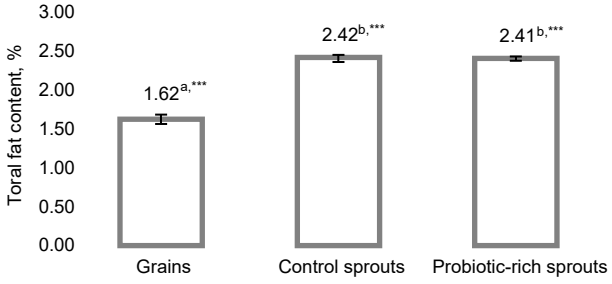

Fig. 1. Total fat content in different forms of buckwheat, %. Mean values with different letters above the bars are statistically significantly different (***, $P < 0.001$)

Fatty acid content and nutritional value of buckwheat seeds and sprouts

Table 2 presents the composition of fatty acids in extracts from lyophilized buckwheat seeds, as well as the control and probiotic-rich sprouts. Germination affected the change of profile and the percentage of individual fatty acids. In total, 13 fatty acids were identified in the seeds and 11 in the sprouts. Myristoleic and pentadecanoic acids were found in the seeds, but not in both types of sprouts. Saturated fats constitute 21.2% in seeds, whereas in sprouts they make up 16.84% (control) and 16.86% (modification), respectively, and unsaturated fats 78.48% ±0.01, respectively; 83.01% ±0.04 and 83.16% ±0.04 of the total fatty acid content.

The highest content of palmitic acid, oleic acid and linoleic acid was found in seeds and sprouts. These results in the case of seeds coincide with the data presented in other scientific studies (Gulpinar et al., 2012; Kim et al., 2004; Taira et al., 1986). It should be noted here that although both the sprouts and the seeds had the highest amount of the above acids, their quantity changed. The content of palmitic acid decreased significantly in control (up to 13.90%) and modified (up to 13.62%) sprouts. In contrast, the linoleic acid content increased both in the control (up to 40.19%) and in the modified form (up to 42.45%).

Table 2. The percentage composition and the ratio of fatty acids in different forms of buckwheat

Fatty acids	Buckwheat forms		
	grains	control sprouts	probiotic--rich sprouts
Saturated			
Myristic acid C14:0	$0.14^{a,**}$ ±0.01	$0.13^{a,**}$ ±0.01	$0.14^{a,**}$ ±0.01
Palmitic acid C16:0	$16.56^{c,*}$ ±0.03	$13.90^{b,*}$ ±0.01	$13.62^{a,*}$ ±0.06
Margaric acid C17:0	$0.31^{b,***}$ ±0.01	$0.07^{a,***}$ ±0.01	$0.08^{a,***}$ ±0.08
Stearic acid C18:0	$2.24^{b,***}$ ±0.02	$1.64^{a,***}$ ±0.02	$1.69^{a,***}$ ±0.03
Arachidic acid C20:0	$1.87^{c,**}$ ±0.01	$1.12^{a,**}$ ±0.01	$1.34^{b,**}$ ±0.02
Monoenoic			
Palmitoleic acid C16:1	$0.40^{a,*}$ ±0.02	$0.57^{b,*}$ ±0.03	$0.60^{b,*}$ ±0.01
Ginkgolic acid C17:1	$0.09^{b,**}$ ±0.01	$0.18^{c,**}$ ±0.01	$0^{a,**}$
Oleic acid C18:1	$39.95^{c,***}$ ±0.1	$36.18^{b,***}$ ±0.09	$33.55^{a,***}$ ±0.11
Eicosenoic acid C20:1	$4.18^{c,***}$ ±0.02	$3.10^{a,***}$ ±0.01	$3.59^{b,***}$ ±0.03
Polyenic			
Linoleic acid C18:2	$32.16^{a,***}$ ±0.11	$40.19^{b,***}$ ±0.15	$42.45^{c,***}$ ±0.08
α-linolenic acid C18:3	$1.64^{a,***}$ ±0.05	$2.84^{b,***}$ ±0.02	$2.93^{b,***}$ ±0.08
Saturated:monoenoic:polyenic			
	1:2.1:1.6	1:2.4:2.6	1:2.2:2.7

Mean values with different letters in the row are statistically significantly different, with significance indicated as follows: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. "±" means standard deviation.

In the literature, lipids of microbial origin are referred to as Single Cell Oil (SCO), and the microorganisms that produce them as oily. Among them we can distinguish bacteria, molds, microalgae and yeasts. Oleaginous microorganisms are defined as being capable of producing and accumulating more than 20% of dry cell substance. They accumulate lipids, which are deposited in so-called lipid droplets (LD). Yeast is the best producer of microbial fat. This is characterized by a fast growth rate and low nutritional requirements. On the other hand, the fatty acid composition can be modified by changing the culture conditions. Not all yeast species are able to synthesize more than 20% of fat in a dry cell substance (Klug and Daum, 2014; Kot et al., 2015; Patel et al., 2020).

Lipid synthesis can proceed in two ways: *ex novo* and *de novo*. *De novo* is based on obtaining fat from acetyl-CoA and malonyl-CoA molecules. On the other hand, *ex novo* from hydrophobic substrates is present in an environment containing phytochemicals, i.e. alkanes, free fatty acids. Subsequently, either directly or after prior hydrolysis, the cells can, for example, be incorporated into biotransformation processes (Kot et al., 2015).

Saccharomyces cerevisiae has not yet been identified in the literature as an oleaginous microorganism. This species is more often referred to as a non-oleaginous yeast. However, there are studies showing their effect on fatty acid production. The mechanisms that stand out here include *de novo* synthesis (Klug and Daum, 2014; Patel et al., 2020).

The fatty acids synthesized by oleaginous microorganisms are primarily myristic, palmitic, stearic, oleic, linoleic, linolenic and palmitoleic acids (Klug and Daum, 2014; Kot et al., 2015). When analyzing the results presented in this publication, it can be seen that there has been a change in some of the fatty acids mentioned. This mainly applies to linoleic and linolenic acids, which, when comparing the control with probiotic-rich sprouts, shows an increase in its amount. Changes in the proportion of individual fatty acids could result from the activity of yeast. This requires further research.

Table 3 shows nutritional information about lyophilisate buckwheat products. The ratio of *n*-6 / *n*-3 fatty acids was higher in seeds than in sprouts. This is related to the change in the fatty acid profile, where the statistically significant amount of saturated acids decreased, while the amount of unsaturated acids increased. Probiotic-rich sprouts contained the most polyene acids. This is associated primarily with an increase in linoleic

Table 3. Nutritional information of lyophilisated buckwheat *Fagopyrum esculentum* Moench, %

Buckwheat forms	Nutrition information							
	SFA	MUFA	PUFA	PUFA/SFA	UFA/SFA	*n*-6 / *n*-3 ratio	IA	IT
Grains	$21.2^{b,***}$ ±0.06	$44.68^{c,***}$ ±0.16	$33.8^{a,***}$ ±0.16	$1.59^{a,***}$ ±0	$3.7^{a,***}$ ±0	$19.68^{b,**}$ ±0.53	$0.22^{b,***}$ ±0	$0.44^{b,***}$ ±0
Control sprouts	$16.84^{a,***}$ ±0	$40.05^{b,***}$ ±0.03	$42.96^{b,***}$ ±0.08	$2.55^{b,***}$ ±0	$4.93^{b,***}$ ±0	$14.17^{a,**}$ ±0.05	$0.17^{a,***}$ ±0	$0.32^{a,***}$ ±0
Probiotic-rich sprouts	$16.86^{a,***}$ ±0.08	$37.8^{a,***}$ ±0.06	$45.38^{c,***}$ ±0	$2.69^{b,***}$ ±0	$4.93^{b,***}$ ±0.02	$14.49^{a,**}$ ±0,45	$0.17^{a,***}$ ±0	$0.32^{a,***}$ ±0

Mean values with different letters in the column are statistically significantly different, with significance indicated as follows: ***, $P < 0.001$; **, $P < 0.01$. "±" means standard deviation.

acid. The PUFA/SFA ratio in probiotic sprouts was higher than in the control sprouts and seeds. Table 2 also shows the ratio of saturated to monoenoic and polyenic acids.

The consumption of *n*-6 fatty acids may be associated with a reduced risk of cardiovascular disease. In the publication by Sacks et al. (2017), it was indicated that saturated fatty acids could be replaced in the diet by polyene acids to prevent cardiovascular disease. The publication by Kim et al. (2004) indicates that during the germination process, the content of polyene acids increases and the number of oleic acid decreases (Kim et al., 2004; Sacks et al., 2017; Wang, 2018).

From a nutritional point of view, lower IA and IT values are considered to be better and such values were observed in this study. It was found that the values of these indicators were lower in sprouts than in seeds.

In the study of Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn., it was shown that during the germination period the content of palmitic acid (14.6%, 15.8%), oleic acid (53.8%, 61.4%), stearic acid increased (2.6%, 6.7%), and the amount of linoleic acid (27.9%, 14.9%), eicosenoic acid (0.8%, 0.6%) decreased (Yiming et al., 2015). However, the study by Guardianelli et al. (2019) examined flour obtained from germinated seeds of *Amaranth caudatus* L. The fatty acid content was tested in amaranth flour: control, germinated for 18 hours (G18) and 24 hours (G24). Referring sample G24 to the control sample, it was noticed that the content of palmitic, linoleic, α-linolenic acid increased significantly and the content of stearic acid significantly decreased (Guardianelli et al., 2019). The above research indicated that the germination process changed the composition of fatty acids, which is similar to the results regarding common buckwheat, which is the subject of this publication.

CONCLUSIONS

During germination, the composition and profile of fatty acids changed. Statistically significant differences are observed between control and probiotic-rich sprouts (palmitic, arachidic, ginkgolic, oleic, eicosenoic, linoleic acid).

Modification with probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* changed the ratio of fatty acids, and increasing the content of polyene acids, both linoleic and alpha-linolenic acid IA and IT, in buckwheat grains and sprouts was considered nutritionally optimal.

Summing up, it can be concluded that buckwheat sprouts modified with the addition of probiotic yeast may constitute a new functional product that can be used in the diet as an element of the prevention of cardiovascular diseases.

To the best of our knowledge, no report has been published so far on pseudocereal sprouts modified by the addition of a probiotic yeast. This study is therefore innovative.

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