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Solid state fermentation (SSF) of lupin flour in obtaining bakery products

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Abstract
Solid state fermentation (SSF) is an effective method used in the food industry in order to improve the nutritional properties of some products, respectively reducing anti-nutritional properties. This paper investigates the possibility of using *Asspergillus sojae* fermented lupin flour added in different proportions (10-30%) in obtaining bakery products with increased nutritional attributes. The proximate composition of the bread obtained with lupin flour fermented with *Aspergillus sojae* showed a higher protein content compared to a similar product obtained from unfermented flour, respectively a lipid intake, mineral substances and total polyphenols more marked by fermentation. The obtained results highlight the possibility of using lupin flour fermented with *Aspergillus sojae* as a floury matrix with applicability in baking in order to increase the nutritional and functional value of the obtained products.
Keywords: *Asspergillus sojae*, nutritional value, polyphenols, elasticity, porosity

1. Introduction

Lupin (*Lupinus polyphyllus)* is a herbaceous plant in the legume family, Fabaceae, which also includes peas. Lupin is a plant cultivated in the Mediterranean basin as food or as an ornamental flower. It is found on the North and South American continents, as well as in the European and African areas of the Mediterranean [1]. Lupin is a plant that is grown for fodder and green manure. Of the more than 200 species, in addition to all wild varieties, there are also species cultivated for human consumption, others for ornamental value, and most as animal feed. The progress of genetics in the 20th century contributed to the complete domestication of lupin species, by hybridizing those with low alkaloid content and those with soft seeds, giving rise to new, sweet varieties, much more suitable for human consumption [2]. Certain varieties of lupin (*Lupinus albus* - white lupin, *Lupinus luteus* - yellow lupin, *Lupinus angustifolius* - blue lupin) are used in food, especially in the Mediterranean area [3].

Aspergillus sojae is a fungal species commonly used in solid-state fermentation (SSF) due to its ability to produce valuable enzymes and bioactive compounds. Here are some key points about its application:

Enzyme Production: Aspergillus sojae is known for producing enzymes like pectinases, which are useful in various industries, including food and biotechnology [4].

Nutritional Enhancement: Aspergillus sojae can enhance the nutritional profile of substrates. It has been shown to increase the phenolic content in rice bran, which can improve its antioxidant properties [5]. Effects of SSF with *Aspergillus sojae,* on

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physicochemical, microbiological and functional properties of canola meal were also investigated with good results [6].

Biomass and Protein Production: Genetic modifications, such as introducing the Vitreoscilla hemoglobin gene, have been used to improve the biomass and protein production of *Aspergillus sojae* in SSF [4].

Industrial Applications: The fungi can be used to produce sterol esters and other bioactive compounds directly from solid substrates without the need for extensive downstream processing [7].

Mould strains belonging to the species *Aspergillus oryzae* and *Aspergillus sojae* are highly valued as koji molds in the traditional preparation of fermented foods such as miso, sake and shoyu, and as protein production hosts in modern industrial processes. *A. oryzae* and *A. sojae* are relatives of the wild molds *Aspergillus flavus* and *Aspergillus parasiticus*. All four species are classified in the group A. flavus. Koji mold species are generally perceived as nontoxic, while wild molds are associated with carcinogenic aflatoxins [8].

2. Materials and method

Lupin flour (LF) produced by Dried Fruits Suppliers was purchased from VitaMix40 shop Timisoara, and wheat flour (WF) type 550 from Auchan supermarket Timisoara. *Aspergillus sojae* were used for fermentation of lupin flour under conditions recommended as optimal for their development. Fermentation was carried out with inoculum in an amount equal to 10% of sample weight and raw material humidity of 60%, in hermetic flasks, incubated at 30 C, until pH reached values in range of 4.0–4.2, for approx. 20–22 h. Three types of composite flours were obtained LFAS 1 (10% lupin flour fermented and 90% wheat flour); LFAS 2 (20% lupin flour fermented and 80% wheat flour); LFAS 3 (30% lupin flour fermented and 70% wheat flour). The bread samples (Figure 1) were obtained as is presented in the figure 2.

The proximate composition was analyzed according to ISO Methods: moisture SR 91/2007 pct.10, protein SR EN ISO 8968-1:2014; total lipid SR 91:2007 pct.14.4; mineral substances SR ISO 2171/2010, sugar SR ISO 91-2007 [9]. The total phenolic content (TPC) of composite flours and breads with different percentages of lupin flour was analyzed according to the modified Folin-Ciocâlteu method [10].

The physical parameters of bread: the volume, the porosity, hight/diameter ratio (H/D) and the elasticity were determined accordingSR91:2007[11].

All results were determined in triplicate and the value reported are the mean of analyzed values. The results were statistically compared with one-way ANOVA, followed by the t-test using Microsoft Excel 365.

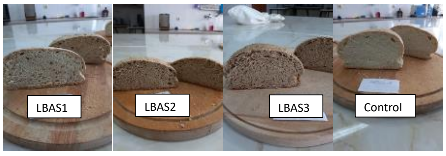

Figure 1. Bread samples fermented with *Aspergillus soja*

3. Results and discussion

The results regarding the proximate composition and TPC of bread samples fermented with *Aspergillus sojae* are presented in the figures 3-4.

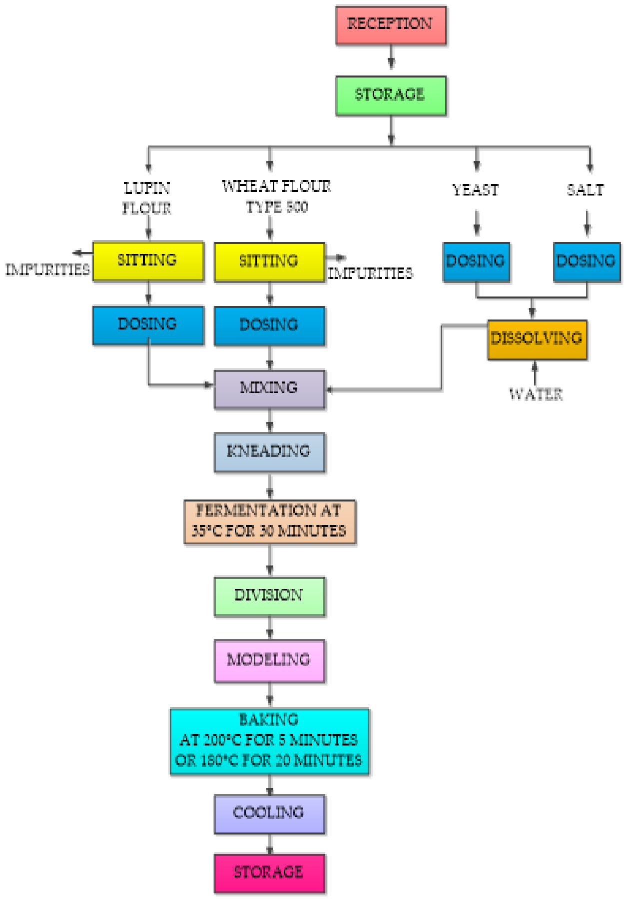

Figure 2. Technological flow of bread making process

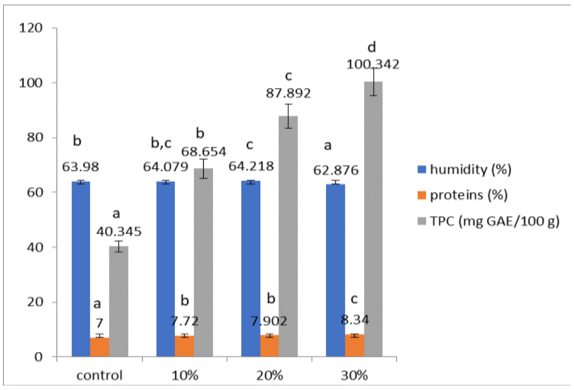

Figure 3. Protein, humidity and TPC of fermented bread

Results are expressed as the mean of three determinations ± standard deviation (SD). Different letters (a-d) indicate statistically significant differences between samples (p < 0.05) according to the t-test.

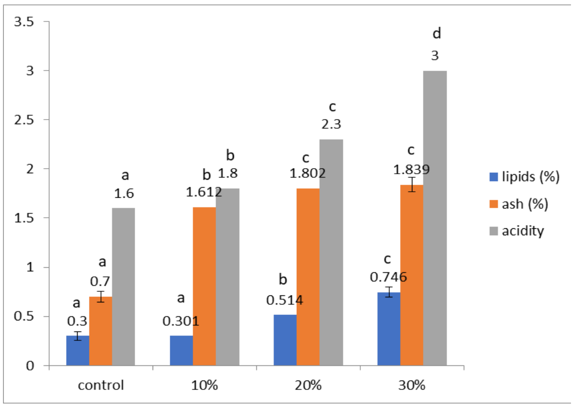

Figure 4. Lipids, ash and acidity of fermented bread

Results are expressed as the mean of three determinations ± standard deviation (SD). Different letters (a-d) indicate statistically significant differences between samples (p < 0.05) according to the t-test.

It can be observed significant increase of lupin protein in fortified samples as effect of fermentation with participation of the *Aspergillus sojae*. In all samples after fermentation, there were higher amounts than before. The protein content in the sample fortified with 30% lupin flour increased with 19.14% compared with the bread without lupin. The same pattern was observed in the case of lipid fraction, where the lupin flour added in percentage of 30% increased the lipid content of bread more then twice compared with the LFAS1 sample (0.746% compared with 0.301%).

The acidity of lupin bread was higher as control and increased with the percentage of lupin flour added in the bread composition.

Regarding mineral substances intake and TPC content, the fermentation improve the bread quality. So, the highest content of TPC was recorded in the case of LFAS3 sample (100.342 mg/100g) more than twice reported to the control (40.345 mg/100g). The increase of total mineral substances, expressed as ash, with the addition of lupin flour in bread recipe was moderate, with an increase of 11.78% for LFAS2 sample, and with 14.08% for LFAS3 sample, reported to the LFAS1 sample.

Opposite, the elasticity and H/D ration of bread decrease by addition of fermented lupin flour (table 1). Higher porosity was observed at addition of 10% fermented flour 68.16% compared with control (66.34%), but the porosity decreased with the addition of 20% fermented lupin flour (61.61%), respectively, the worst porosity was recorded at the addition of 30% fermented lupin flour (47.88%). The elasticity of bread registered the same decreasing trend, with a decrease between 4.21-24.21% depending of percentage of fermented lupin added and compared with the control.

The volume of bread samples decreased with addition of fermented lupin flour, the smallest value was recorded for LFAS3 sample and the highest for control. H/D value did not register significant differences between the samples and was not affected too much by the addition of fermented lupin flour.

The same trend was observed in the case of bread with the addition of unfermented lupin flour in wheat flour [12]. A decrease in volume between 0.69-7.47% compared to the control was observed when adding between 10-30% of lupin flour. The porosity registered the largest decrease, being 35.26% compared to the control when 30% lupin flour was added to the bread [12].

Compared to bread with 30% added fermented lupine flour with *Aspergillus sojae*, when the elasticity decreased with 24.21%, it is observed that in this case, the fermentation processes have a positive role by reducing less the elasticity of the bread compared to the case of the addition of unfermented flour.

The maximum decrease in elasticity reported in the case of bread with unfermented lupin flour was 11.27% compared to the control [12], for the sample with 30% addition, while in the case of the addition of the same percentage of fermented lupine flour, the decrease in elasticity was 24.21% compared to the control.

We can therefore say that the fermentation of lupine flour leads to a better porosity, but worse elasticity of the bread compared to the same addition of unfermented lupin flour in bread. For the H/D ratio, no significantly different values were recorded in the case of the addition of unfermented lupin flour [12].

The nutritional enrichment of bread by fortification with baobab or Moringa flour has also been reported in other studies [13,14]. It has been observed that the fortification of bread from wheat flour in a proportion of up to 30% baobab flour improves the nutritional properties and active principles of the bread, but decreases the physical properties, especially the elasticity, porosity and volume of the bread.

Table 1. Physical parameters of fermented bread

Sample	Volume (cm^3/100 g)	H/D	Porosity (%)	Elasticity (%)
Control	840	0.535	66.34	95
LFAS 1	770	0.477	68.16	91
LFAS 2	760	0.450	61.61	81
LFAS 3	600	0.535	47.88	72

Conclusions

The study indicated that SSF of lupin flour with A. sojae can improve the nutritional and functional properties of bread,, but some shortcomings in the appearance of bread can be observed. Despite this, solid-state fermented lupin flour is a promising ingredient and can find potential industrial applications in the development of novel functional bakery products.

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