SUMMARY OF PhD THESIS

Research on exploiting the bioactive potential of ancient wheat species for obtaining sourdough and nutritionally optimized bread

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1. INTRODUCTION

Currently, consumers tend to direct their attention to the so-called "foods as medicine", i.e., functional products that help to control and prevent chronic diseases. Bread is recognized as an important source of carbohydrates, key macronutrients in providing energy, but also for bioactive compounds (antioxidants, vitamins, minerals) with a beneficial effect on health. However, due to its high glycemic index (95) and low fiber content, white bread made from refined grains has started to be replaced by more nutritionally advantageous products. Thus, sourdough fermented bread began to receive more and more credit in the global bakery market due to its superior sensory quality, lower glycemic index (54), high content of bioactive compounds, and resistant starch.

Although the main purpose of sourdough is to ferment the flour, it is also used to obtain other attributes, such as increasing the shelf life or improving the products' nutritional, sensory/aromatic, and rheological profile. Also, the technological parameters of dough processing and endogenous factors in flour are important in influencing the microbial community. The complexity of the dough is given by the interaction between bacteria and yeasts with the rest of the ingredients, depending on the technological parameters.

On the other hand, ancient cereals such as einkorn (*Triticum monococcum* L. ssp. *Monococcum*), spelt (*Triticum aestivum* L. ssp. *Spelta*) or emmer (*Triticum turgidum* L. ssp. *Dicoccum*) have started to return in the attention of consumers due to this specific chemical composition (proteins, fibers, unsaturated fatty acids, minerals, vitamins and other bioactive compounds) that have health benefits. Moreover, ancient grains show a higher resistance to fungal diseases compared to common wheat (*Triticum aestivum* L.), which makes them more suitable for organic production where the use of pesticides and fertilizers is prohibited. Also, their cultivation does not require advanced technology, and by obtaining ecological grains, soil biodiversity is preserved.

The present study aims to obtain a bakery product with superior/enhanced nutritional properties that exhibit a lower digestion rate and a slower rate of starch breakdown so that it can help control blood sugar levels. In this sense, three types of ancient cereals (einkorn, emmer, spelt) were chosen, which will represent the raw material in obtaining some assortments of sourdough bread.

2. PURPOSE AND OBJECTIVES

The purpose of this thesis entitled "Research on the exploitation of the bioactive potential of ancient wheat species for obtaining sourdough and nutritionally optimized bread " consists in the exploitation of the biologically active potential of some selected species of ancient wheat (spelt, einkorn and emmer) in order to obtain sourdough and bread varieties rich in fiber and with improved nutritional properties.

In order to achieve the proposed goal, three research directions were established, considered to be major research objectives. In turn, these were divided into several sub-objectives as follows:

I. The first research direction – Evaluation of the influence of lactic acid bacteria on the fermentation process of sourdoughs obtained from einkorn, spelt and emmer flour.

- I.1 Evaluation of the microbial development dynamics of the strain of *Lb. plantarum* ATCC 8014 on the matrix represented by ancient wheat flours;
- I.2. Evaluation and monitoring of quality parameters specific to sourdoughs (pH, TTA, rheological parameters, flavor compounds);
- I.3 Evaluation of the main bioactive compounds in sourdough.

II. The second direction of research – Obtaining and characterizing some types of bread from einkorn, spelt and emmer flours, rich in fiber and with improved nutritional characteristics.

- II.1 Evaluation of the main bioactive compounds from bread samples obtained with sourdough;
- II.2 Quantification of volatile derivatives and aroma profile analysis of bread samples;
- II.3 Textural and sensory analysis of bread samples.

III. The third direction of research – *In vitro* digestibility evaluation of carbohydrates from bread and quantification of the digestibility index.

- III.1 Quantification of starch, maltose, glucose and fructose contents of sourdough bread samples;
- III.2 Determination of the *in vitro* digestibility index (IVD).

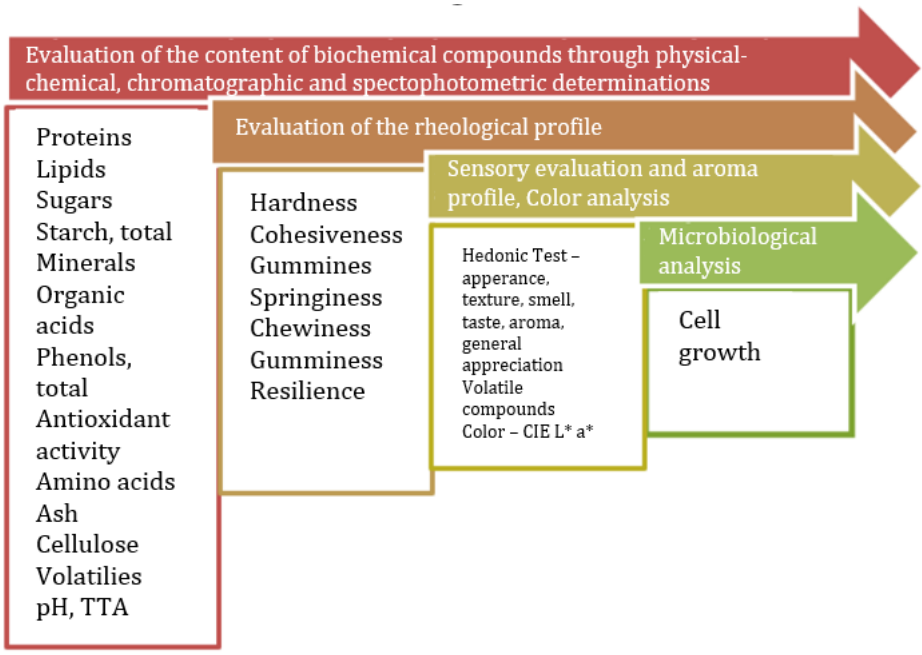

Fig.1. Experimental design regarding the content of biochimic compounds, rheological properties, and sensorial profile in raw materials, soudoughs and final products (original)

3. THESIS STRUCTURE

The present thesis "Research on the exploitation of the bioactive potential of ancient wheat species for obtaining sourdough and nutritionally optimized bread" is structured in two main parts.

The first part called "The current state of knowledge regarding the chemical composition of the raw materials used and the production of sourdough" is composed of two chapters during which the materials used in the recipe for the manufacture of the final product are characterized, as well as notions regarding the method of obtaining the sourdough and the actions it exerts on the other constituents of the bakery product.

The second part of the paper provides "Own contributions in the development of a bakery product with improved/functional nutritional qualities with the help of sourdough", and is structured in five chapters including personal contributions, results, discussions, conclusions and research perspectives.

4. PERSONAL CONTRIBUTION

4.1. Material and method

The experiments were carried out in the Pilot Plant for obtaining bakery products from the Faculty of Food Science and Technology, the Food Quality Control laboratories, the Microbiology laboratory, the Phytochemical Analysis and Agro-alimentary Metabolomics laboratory from the Institute of Life Sciences, USAMV Cluj-Napoca, the Interdisciplinary Research Platform within the University of Life Sciences "King Mihai I" Timişoara and the Institute of Food Engineering within the Polytechnic University of Valencia, Spain.

Thus, the experiment assumed in the first phase the obtaining of 4 variants of sourdough from the 4 types of flour used in the study, namely, with: whole wheat flour (M_0), einkorn (M_1), spelt (M_2) and emmer (M_3). From the 4 variants of sourdough that were fermented by the strain of *Lactobacillus plantarum* ATCC 8014 at 30°C, samples were taken at time 0 of fermentation, after 12 hours and after 24 hours in order to analyze how the duration of fermentation of sourdough influences the final product both nutritional, rheological and sensorial. As a result, in the second stage, each type of sourdough was used to obtain a complementary type of bread (common wheat bread – P_0, einkorn – P_1, spelt – P_2 and emmer – P_3) in terms of the flour used.

The biological material used was composed of: whole wheat flour, whole rye flour, whole einkorn flour, whole spelt flour, whole emmer flour, culture of lactic acid bacteria (*Lb. plantarum* ATCC 8014), yeast, Mung beans , inulin, sugar, sunflower oil and salt. Culture of *Lb. plantarum* ATCC 8014 was purchased from Microbiologics (Minnesota, USA). With the exception of whole emmer flour, all other types of flour used were purchased from the organic farm BioFarmland Manufactura S.R.L (Arad, Firiteaz, no. 350). The rest of the ingredients used come from specialized stores in Cluj-Napoca.

4.2. Results and discussions

Analyzes were performed in triplicate, lowercase letters indicating statistically significant differences between the 4 types of sourdough/bread at the same time (0, 12, 24 H), and uppercase letters indicating statistically significant differences between the 4 types of sourdough/bread at different moments (0, 12, 24 H). For the statistical processing of the obtained data, the test of multiple correlations was used - the Duncan test. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) using Heatmap were also performed.

4.2.1. Results regarding the first research direction

From the point of view of cell growth, the four types of flour showed a significant cell growth during the three fermentation times (0, 12, 24 H), which proves a good adaptability of the strain *Lactobacillus plantarum* ATCC 8014 on these vegetables matrix.

The measured values of pH (below 4 after 24H) and total titratable acidity (15.6 - 23.4 mL of NaOH/10g after 24H) were optimal for sourdough, comparable to those quoted in the literature.

The organic acids that are formed as a result of lactic fermentation had a progressive increase during the 24 hours, especially lactic acid (M_1/einkorn – 6.37 mg/g, M2/spelt – 5.43 mg/g).

Regarding sugars, glucose followed an upward trajectory with increasing fermentation time for ancient flours (M_1/einkorn – 4.99 mg/g, M_2/spelt – 5.36 mg/g, M_3/emmer – 2.71 mg /g), while fructose decreased with fermentation time. Maltose also decreased as a result of its conversion to glucose and consumption during the fermentation process.

The concentration of crude cellulose in all sourdough samples decreased during fermentation as a result of the process of solubilizing insoluble fibers into soluble fibers, recording values between 1.05 and 2.09% in the fermented variants for 24 hours.

Fermentation increased mineral levels as a result of acidification that indirectly activated endogenous wheat phytases, as well as due to microbial enzyme activity. Thus, sourdough with einkorn/M_1 stood out for its content in zinc, potassium, iron and calcium; and sourdough with spelt wheat/M_2 by its magnesium content. In contrast, emmer sourdough had the lowest mineral levels.

The content of polyphenols in sourdough samples increased with increasing fermentation time, acidification leading to improved extractability of these compounds. Among the ancient flours, the sourdough variants obtained from einkorn/M_1 stand out (from 118 mg GAE/100g to 167 mg GAE/100g). The antioxidant activity was closely related to the polyphenol content of the samples.

42 volatile compounds were detected that accumulated as a result of acidification and the release of flavor precursors such as amino acids. The most representative flavor compounds that gave sourdough a pleasant, sweet, fruity flavor were 1-hexanol, 1-pentanol, hexanal, benzaldehyde, acetophenone, hexanoic acid, and 2-pentyl furan.

The impact of fermentation on the rheological properties of sourdough was established with the help of the elastic modulus – G'; and of the plastic modulus G". Thus, it was noted that the elastic modulus was higher than the plastic modulus, which leads to the conclusion that sourdough has an elastic behavior.

4.2.2. Results regarding the second research direction

The protein content of the 12 types of bread was variable. Amino acids, on the other hand, followed an upward trajectory as a result of the increase in the duration of its fermentation. The best levels being reported in sample P_2/bread with spelt flour – 111.1 mg alanine/100g sample.

Lipids showed low values in all samples, possibly attributed to the fermentation of microorganisms that use lipids as an energy source for their metabolic activities.

Among the organic acids, lactic acid was noted with increased values in breads with einkorn/P_1 (1.13 mg/g) and emmer wheat/P_3 (0.95 mg/g) and citric acid again in the sample with einkorn/P_1 (0.55 mg/g). Acetic acid showed low amounts in all samples. The importance of organic acids is given by their ability to stop the appearance of pathogenic bacteria, respectively to extend the shelf life of products.

The ash content of the bread variants increased with increasing sourdough fermentation time, suggesting that fermentation increases the levels of minerals. Thus, breads with ancient flours such as sourdough were noted for their high levels of minerals such as zinc, potassium, manganese, calcium, and iron.

Sourdough positively influenced the levels of phenolic compounds in bread as a result of weakening the dough matrix and releasing a large number of extractable polyphenols. The best results were recorded in the P_1/einkorn sample (140 – 149 mg GAE/100g), and the lowest in the P_3/emmer sample (120 – 125 mg GAE/100g). The antioxidant activity of bread is closely related to the content of polyphenols, so the samples followed the same trajectory.

24 volatile compounds were determined in bread variants with modern and ancient wheat flours. Among the most representative for bread are: 2-methyl-1-butanol, 2-heptanone, hexanal and 2-pentylfuran. As in the case of sourdough, alcohols and aldehydes dominated in bread. An explanation for their presence, especially of hexanal, could be the degradative oxidation of the unsaturated fatty acids that contribute to its formation.

In terms of textural parameters; hardness, gumminess, chewiness and springiness index were positively and significantly influenced by fermentation time. On cohesiveness and resilience, fermentation time had no significant influence, but their values increased with fermentation time. The quality and quantity of gluten significantly influence the textural parameters of the bread. Ancient flours produce softer doughs with less elasticity and greater extensibility compared to modern wheat.

The color parameters of the samples were not significantly influenced by the sourdough fermentation time. Prolonged fermentation periods leading to their growth. In general, the sample with emmer/P_3 had the highest values for brightness, red and yellow tones, followed by the sample with einkorn/P_1.

The sensory analysis - Hedonic Test - of the finished products showed that the bread variants in which sourdough fermented for 12 hours was used are the most appreciated by the evaluators in terms of appearance, taste and texture. From the point of view of smell and aroma, a significant decrease can be seen with increasing time of yeast fermentation (24 hours) in all samples.

4.2.3. Results regarding the third research direction

Starch reached maximum values in all types of bread fermented with sourdough fermented for 12 hours (34.3 – 39.6 g/100g). The high values recorded for this fermentation time could be due to the differences between the starch granules in terms of size and degree of incorporation into the protein matrix. Also according to studies, its digestion rate decreases as a result of the production of organic acids, and sourdough bread contains a greater amount of resistant starch, a harder-to-digest form of starch. These aspects lead to the conclusion that the time in which the body can break it down increases significantly, which causes the blood sugar level to be maintained for a longer period.

In vitro digestion of the final products revealed that there is a decrease in the amount of maltose, glucose, fructose with the decrease in the fermentation time of sourdough. Thus, in the samples in which sourdough with 24 hours of fermentation was used, the highest value in the maltose content was recorded in the control sample/P_0 with 184.80 mg/g and the lowest in the sample with emmer flour/P_3 with 137.08 mg/g. Regarding the fructose content, in the first sample/P_0 a level of 29.93 mg/g of this carbohydrate was determined, and in the last/P_3 a quantity of only 21.11 mg/g. While for glucose the highest values (after 24 hours) were recorded in samples P_1/einkorn – 12.90 mg/g and P_0/common wheat – 13.13 mg/g, and the lowest again in sample P3/emmer – 10.02 mg/g. Also, the residues resulting from digestion contain appreciable amounts of these monosaccharides, especially maltose. Regarding the degree of digestibility in vitro (IDV), it increased with the duration of fermentation. The highest rate was recorded in the sample with emmer/P_3 flour (86.5% 24 H).

As a conclusion, the levels of carbohydrates recorded in the bread samples lead to the hypothesis that breads obtained from ancient flours, fermented with sourdoughs from ancient flours and *Lactobacillus plantarum* ATCC 8014, and to which other ingredients intended to decrease glycemic index (inulin) may have some potential in lowering blood glucose levels.

5. CONCLUSION AND RECOMMENDATIONS

Based on the results obtained in sourdoughs, it can be stated that *Lactobacillus plantarum* ATCC 8014 shows good adaptability, cell growth and a good acidification rate, in sourdough obtained with wheat flour, einkorn flour, spelt flour and emmer flour, between ancient wheat sourdough and common wheat sourdough being clear differences. Fermentation-specific metabolites were identified as a result of the interaction between ancient flours and *Lb. plantarum* ATCC 8014. Among the ancient cereals, emmer wheat showed a distinctive behavior during fermentation in terms of cell dynamics, carbohydrate metabolism and lactic acid formation. This could be explained by its higher content in resistant starch, but also due to the lower amounts in zinc and manganese, important factors for the propagation of microbial cells. Cluster analysis showed specific volatile compounds for each type of sourdough.

Regarding the final products (bread), the statistical analyzes of the obtained data indicated clear differences between the samples obtained from modern varieties and those obtained from ancient varieties. Both the specific chemical composition of flours and sourdough fermentation significantly influenced the nutritional, textural and sensory properties of breads. Sour dough fermentation improved the content in bioactive compounds (minerals, polyphenols), to the same extent that they improved the textural and sensory characteristics. Also, the *in vitro* digestibility of the bread samples had typical values for bread with a high fiber content, and even if the fructose content was higher in the digested fractions than the glucose content, the concentration is not too high for a rational diet. However, as with sourdough, the emmer wheat bread variants showed statistically significant differences from the rest of the grains. Emmer wheat bread presented the highest digestibility, content in volatile compounds, as well as textural parameters.

5.1. Originality elements of the thesis

The elements of originality of the thesis include the following:

- characterization and comparison of the main ancient wheat species (*Triticum monococcum* – einkorn, *Triticum spelta* – spelta, *Triticum dicoccum* – emmer) from the point of view of chemical composition;
- characterization of the fermentation process of sour doughs obtained from the flours of ancient wheat species;

- determination of the influence of lactic fermentation on the content of bioactive compounds and volatile compounds in sour doughs with the help of HPLC-RID, AAS, ITEX/GC-MS techniques;
- characterization of sourdough from a rheological point of view;
- the use of acid doughs in obtaining innovative final products with functional potential;
- characterization of final products with the help of physicochemical (macronutrients), spectrophotometric (bioactive compounds, volatile compounds), rheological, colorimetric and sensory determinations;
- *in vitro* digestion of final products obtained by fermentation with acid doughs and studying its influence on the content of reducing sugars (maltose, glucose, fructose).

5.2. Prospects

Based on the results obtained, the following prospects for the future are proposed:

- determining the glycemic index of sourdough fermented bread and determining the degree to which it is influenced by lactic fermentation and the chemical composition (ex: fibers) of ancient flours (einkorn, spelt, emmer);
- conducting clinical studies in order to determine the glycemic response as a result of the consumption of fiber-enriched bread, obtained from einkorn, emmer, spelt, rye using the indirect method;
- the use of other microorganisms (*Lactobacillus sanfranciscensis*, *Weissella confusa*) in the fermentation of ancient flours with the aim of identifying a key strain of microorganisms to maximize the content of certain bioactive compounds;
- conducting chemical analyzes to deepen and compare the bioactive potential of modern and ancient wheat species;
- clearer demarcation (physicochemical determinations, advanced analysis techniques) between einkorn, spelt and emmer flours in terms of nutritional value.