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FINE-TUNED EVALUATION OF SELECTED RHEOLOGICAL PROPERTIES OF COMPOSITE FLOURS WITH THE ADDITION OF NATURAL POLYSACCHARIDES

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ABSTRACT

As part of the research, the addition of extracts from medicinal mushrooms (Maitake, Shitake, Cordyceps and Reishi), oat β-glucan extract and yeast extract with a high proportion of polysaccharides to composite flours intended for bread preparation was verified, which brings nutritional or economic benefits to this basic food. In composite flours, in which additives of 5%, 10% and 15% were indicated, their rheological behaviour and water absorption were monitored. The addition of medicinal mushroom extracts reduced the water absorption values by up to 25% depending on the amount of the additive, compared to the control. On the contrary, the addition of oat β-glucan extract and yeast extract in water absorption increased by up to 16% and 54%, respectively. Such composite flours are suitable from the point of view of the economy of production of final products, since more loaves of bread can be prepared compared to control flour. The evaluation of rheological properties on the Mixolab 2 device, Chopin technologies, confirmed the non-standard course of the recorded curves. The evaluated parameters expressed by the Mixolab software were compared with the evaluation performed manually using the conventional Farinograph®Brabender method. Due to the non-standard course of the Mixolab curves, the manual evaluation can be considered as an additional interpretation framework that allows a better description of the non-standard behaviour of the curves, accounting for individual variations the emergence of two peaks on the curve. From the point of view of the rheological properties of the doughs and their processability, it is possible to recommend additives containing polysaccharides: medicinal mushroom extracts (Shiitake, maitake, Cordyceps, Reishi), oat β-glucan extract and yeast extract up to 15%, with the exception of maitake extract, for which an addition of up to 10% is acceptable.

Keywords: extracts of medicinal mushrooms, oat β-glucan extract, yeast extract, rheology

INTRODUCTION

Interest in improving the health benefits of cereal foods is constantly increasing, also due to the high frequency of their consumption around the world and the wide possibility of their use as a carrier of health-promoting dietary components. Bread is a commonly consumed food, the nutritional value of which can be relatively easily improved by adding ingredients containing biologically valuable substances. If these valuable components are polysaccharides, they have received considerable attention due to their physiological properties, including immunomodulatory, antioxidant, antiviral, anticarcinogenic and anti-inflammatory effects (**Rathore, Prasad and Sharma, 2017; Niego *et al.* 2021; Sengul *et al.*, 2022**). From a nutritional point of view, significant attention is paid primarily to non-starch polysaccharides, especially glucans.

Glucans are part of a group of biologically active natural molecules and are constantly gaining a lot of attention not only as an important dietary supplement, but also as an immunostimulant and potential drug (**Twig *et al.*, 2019; Garcia *et al.*, 2022**). An immunomodulator is defined as a substance capable of interacting with the immune system, and this group includes a number of molecules, mainly natural ones (curcumin, resveratrol, ellagic acid, etc.), however, glucan has the best position among other immunomodulators (**Vetvicka *et al.*, 2019**).

β-(1→3)-D, β-(1→4)-D glucans (β-D-glucans) are found in larger quantities in the cell walls of some cereals, where they make up to 30% of the dry matter of non-starch polysaccharides. Due to their physicochemical properties, β-D-glucans have therapeutic and nutritional potential and a specific place for their functional properties in various food formulations (**Daou *et al.*, 2012; Hu *et al.*, 2013; Ahmad and Kaleem, 2018; Kaur *et al.*, 2019; Caseiro *et al.*, 2022; Jurkaninová *et al.*, 2024**). Related polymers β-(1→3)-D, β-(1→6)-D glucans, or β-glucans, with mixed bonds, are also synthesized by higher fungi, yeasts and moulds (**Velíšek and Hajšlová, 2009; Zhu *et al.*, 2016**). Thus, β-glucan is an important component of fibre, which can be extracted from oats, barley, bacteria, yeasts and fungi. In addition to its nutritional benefits, its potential in the food industry as a stabilizer, fat replacement, water-binding agent, oil retention agent, and whipping agent has also been demonstrated (**Ahmad and Kaleem, 2018**). Undoubtedly, it is also necessary to consider it as an important commodity, because on a global scale, the sales of plant and mushroom β-glucans are around USD 400 million (**Jurkaninová *et al.*, 2024**). However, cereal β-D-glucans have specific molecular-structural properties that distinguish them in terms of molecular weight, degree of branching and types of bonds from other important sources of β-D-glucans, e.g. from fungi and yeasts. Their nutritional relevance is also supported by approved health claims, (i) in terms of lowering serum glucose levels, provided that at least 4 g of β-D-glucans from oats or barley per 30 g of available carbohydrates are consumed with food, (ii) and in relation to the reduction of LDL cholesterol when consuming high molecular weight oat β-D-glucans at a rate of 3 g per day in solid food (**FDA, 1997; FDA, 2004**).

In recent years, there has been an exponential increase in research on fungal polysaccharides due to their unique biological properties and applicability in various fields. In addition, mushrooms to produce polysaccharides can be grown in a sustainable way (**Elisashvili, 2012**; **Wasser, 2014; Su *et al.*, 2016**). β-(1→3)-D, β-(1→6)-D glucans are part of water-soluble polysaccharides (WSP) and are considered the main bioactive polysaccharides in fungi. They are credited with anti-tumour activity, and their chemical modification is often carried out to improve this activity (**Wasser, 2002; Garcia *et al.*, 2022; Wang *et al.*, 2022**). Fungal polysaccharides prevent oncogenesis, show direct antitumor activity against various allogeneic and syngeneic tumours, and prevent tumour metastasis. They produce their antitumor effects by activating various immune responses (**Wasser, 2002; Chen *et al.*, 2005**), however, the exact immunomodulatory and anticancer mechanisms of action have not yet been revealed (**Garcia *et al.*, 2022**). As for the antioxidant ability to scavenge free radicals, there are significant differences between mushroom polysaccharides. The strongest ability to scavenge hydroxyl radicals has been found in Shiitake polysaccharides (*Lentinula edodes*). Reishi (*Ganoderma lucidum*) polysaccharides, in turn, have the highest inhibitory activity for tumour cell proliferation (**Chen, 2015**). The author confirmed that of the mushroom polysaccharides evaluated by him, polysaccharides from *Hericium erinaceus*, *Lentinula edoles* and *Ganoderma lucidum* have the potential to be used as valuable food additives or sources of therapeutic agents for antioxidant and cancer treatment.

Yeast as a by-product is also an important source of β-glucans with already proven biological and technological properties. For use in food and beverages, β-glucan must be extracted, purified, and fully characterized (**Gautério *et al.*, 2022**). The source for the isolation of β-glucan is various yeasts, but those that are considered

waste from the brewing industry have the potential. Despite the fact that a large amount of brewer's yeast is used as feed, the β-glucan isolated from them can achieve a higher commercial value and maximize the overall profitability of the brewing process (**Zechner-Krpan *et al.,* 2009**). Yeast β-glucans are considered safe food ingredients by the European Food Safety Authority (**EFSA, 2011**) and commercial products are available.
Consumers are increasingly aware of the relationship between nutrition and health. Currently, innovations in bread mainly focus on the nutritional improvement of bread by enriching or using different flours. However, the application of non-bakery additives, including extracts containing polysaccharides of various origins, carries several technological risks in the production of bread. Bread dough is a versatile matrix that provides a variety of bread products after rising and baking. Kneading is one of the key steps that determine the mechanical properties of the dough, which have a direct impact on the quality of the final product. Kneading evenly distributes the different ingredients, moisturizes the components of the wheat flour, supplies the necessary mechanical energy to develop the protein network, and incorporates air bubbles into the dough (**Rossel, 2011; Delcour *et al.,* 2012; Shewry, 2023**). Bread dough is a viscoelastic material that exhibits an intermediate rheological behaviour between a viscous liquid and an elastic solid. The rheological properties of wheat flour dough are largely driven by the contribution of starch, protein and water. The protein phase of flour has the ability to form gluten, a continuous macromolecular viscoelastic network, but only if a sufficient amount of water is provided for hydration and a sufficient supply of mechanical energy during mixing (**Veraverbeke and Delcour, 2002; Don, 2022; Yang *et al*., 2022; Wieser *et al*., 2022**). By adding water and kneading, the gluten matrices in the individual cells combine to form a network with the input of mechanical energy and increase the hydration of the gluten protein. The amount of water absorbed by flour (WA) is a key feature used to optimize mixing conditions. WA is determined by the amount of water absorbed by the individual components of the grain, with starch, protein and fibre having the greatest effects (**Shewry, 2023**).
The addition of non-bakery ingredients when applied to a basic cereal-based food, i.e. bread, significantly affects the preparation of bread, from the very beginning of this process (for example the amount of water that is added to the flour to create a dough with the desired consistency). Non-starchy polysaccharides (NSPs) affect the rheological properties of dough and cause an increase in water absorption and a reduction in dough development time (**Shyama Prasad Rao *et al*., 2007**). Among NSPs, arabinoxylans and β-glucans play the most important role in bread production. Their addition in amounts up to about 6% causes an increase in the absorption of water in the flour and, as a result, forces the use of a greater addition of water to achieve the desired consistency of the dough (**Bieniek and Buksa, 2023**). β-glucan, as a highly hydrophilic non-starch polysaccharide, is known for its ability to alter functional characteristics such as viscosity, rheology, texture, and sensory properties of a food product (**Kaur *et al.,* 2019; Gautério *et al.,* 2022**).
The aim of the research presented in the article was to trace the effect of polysaccharides of various origins (from oats, mushrooms, yeast) processed into commercially available extracts, on the rheological properties of dough as a process intermediate in bread production. The scientific hypothesis assumed a significant effect of these components on water absorption, the weight of the prepared dough and on the course of curves recorded by special devices, or even on their non-standard course. Another contribution of this study lies in the methodological refinement of Mixolab curve interpretation for non-standard dough systems, supported by a comparative dataset of polysaccharide-enriched composite flours. The objective was to establish a robust evaluation framework for these complex matrices.

MATERIAL AND METHODS

Used material and experiment conception

In the presented part of the research, several types of natural polysaccharide extracts were applied to T650 wheat flour (Mlyn Zrno, Veľké Hoste, Slovak Republic, the quality of which is defined by the following parameters: energy value 1464 kJ, carbohydrate content 71 g/100 g, including sugars 1.59 g/100 g, fibre content 3.3 g/100 g, protein content 11 g/100 g, fat content 1.3 g/100 g, including saturated fat 0.3 g/100 g, salt 0.01 g/100 g, and ash content 0.65 %): namely, extracts from Shiitake mushrooms (*Lentitula edodes*) (**Shi_5, Shi_10, Shi_15**), Maitake (*Grifola frondosa*) **(Mai_5, Mai_10, Mai_15**), Cordyceps (*Copdyceps sinensis*) (**Cor_5, Cor_10, Cor_15**) and Reishi (*Ganoderma lucidum*) (**Rei_5, Rei_10, Rei_15**) with a polysaccharide content of 30%; oat β-glucan (**OaE)** and yeast extract (**YeE**) with a high content of β-glucan (70% and 80%, respectively). The composition of the natural polysaccharide extract is standardized and declared by the manufacturer, which can be considered an advantage in terms of reproducibility and practical application. The declared composition is presented in Table 1. The commercially available extracts used were supplied from the source: One Pharma s.r.o, Janka Kráľa 5, 974 01 Banská Bystrica, Slovakia, origin Natural Field (Xi'an Natural Field Bio-Technique Co., Ltd., Xi'an, China), respectively Chemopharma Vídeň, s.r.o., Pod Parkem 1441, 543 01 Vrchlabí, Czech Republic, and meet the conditions regarding the content of elements and microbiological requirements.

Table 1 Composition of the medicinal mushroom extracts and natural polysaccharide extracts (as specified by the manufacturer)

Extracts	**Shiitake mushroom extract**	**Maitake mushroom extract**	**Cordyceps extract**	**Reishi mushroom extract**
Part used	Upper part	Aerial part	Whole	Whole
Analysis item				
Description	Brown-yellow powder	Brown powder	Light brown-yellow powder	Brown-yellow powder
NTL (polysaccharides) (g/kg)	305	305	305	311
Particle size (mesh)	80	80	80	80
Ash (g/kg)	37.4	32.7	32.2	33.5
Heavy metals	≤ 10ppm	≤ 10ppm	≤ 10ppm	≤ 10ppm
Benzo(a)pyrene	≤ 10ppb	≤ 10ppb	≤ 10ppb	≤ 10ppb
PAH 4	≤ 50ppb	≤ 50ppb	≤ 50ppb	≤ 50ppb
Extracts	**Oat beta-glucan**		**Yeast extract**	
Analysis item	Specification	Result	Specification	Result
Description	Fine powder, white to light yellow	Conforms	White/off-white powder of characteristic odour	Conforms
Beta glucans (g/kg)	≥ 700	728,0	≥ 800	808,2
Water content/loss on drying (%)	≤ 5	0.57	5	4.12
Ash (g/kg)	≤ 80	23,4	≤ 70	21,5
Microbiological tests				
Total plate cound (cfu/g)	≤ 100000	Conforms	≤ 10000	Conforms
Mold (cfu/g)	≤ 100	Conforms	≤ 100	Conforms
Coliforms (cfu/g)	≤ 100	Conforms	≤ 100	Conforms

With their application, composite flours were prepared with the addition of selected natural polysaccharide extracts (medicinal mushroom extracts (**MuE**), an extract containing oat β-glucans (**OaE**) and a yeast extract containing β-glucan (**YeE**), and control (wheat flour T650, **WF**) in the variants listed in Table 2.

Table 2 Combinations of evaluated composite flour (experimental design)

Sample	Wheat flour T650, WF	Medicinal mushrooms extracts, MuE	Oat β-glucan extract, OaE	Yeast extract, Yee
WF	100%	–	–	–
MuE_5	95%	5%	–	–
OaE _5	95%	–	5%	–
YeE _5	95%	–	–	5%
MuE _10	90%	10%	–	–
OaE _10	90%	–	10%	–
YeE _10	90%	–	–	10%
MuE _15	85%	15%	–	–
OaE _15	85%	–	15%	–
YeE _15	85%	–	–	15%

Legend: WF [Wheat flour T650], MuE [Medicinal mushrooms extracts], OaE [Oat β-glucan extract], YeE [Yeast extract], 5/10/15 [amount of addition 5%, 10%, 15%]

Rheological measurements

Composite flours (Table 2) were analysed for selected rheological properties on the Mixolab 2 device (Chopin Technologies, Villeneuve-la-Garenne, France) according to ICC Standard Method 173 (**ICC, 2011**). The device allows the investigation of rheological characteristics by measuring in real time the torque (expressed in Nm) produced by the dough between the two mixing blades. Mixolab simulator (S-protocol) has been specifically developed to get results comparable (values and units) to Farinograph data. Analysis conditions: constant test temperature 30 °C; test time 30 minutes; running speed 80 rpm; target value 1.1 Nm. The evaluated parameters from the mixolab curves were water absorption (WA, %); dough development time (DDT, min.); dough stability (DS, mixing resistance of dough, min.) and degree of softening (SOF, 12 min. after max).

In addition to standard Mixolab software output, a secondary interpretative evaluation of the torque curves was performed. This manual assessment was applied specifically to samples exhibiting non-standard dual-peak behaviour, which is not fully resolved by the standard software algorithm. This approach represents an interpretative analytical framework based on farinograph principles rather than a repeated instrumental measurement, and therefore does not constitute an independent replicate dataset in the statistical sense.

The evaluation was performed by visual human assessment based on the classical methodology for the evaluation of farinographic curves (**Brabender® Farinograf, 2022, 2024**): DDS is measured at the point of the upper maximum of the curve; The DS is defined by points on the top line of the curve that intersect the maximum consistency given by the centre of the curve; The degree of softening is measured at the 12th minute after maximum in the middle of the curve. The manual interpretation of Mixolab curves was applied as an auxiliary analytical approach aimed at improving the interpretability of non-standard dual-peak torque profiles. This approach does not replace standard software evaluation, but complements it in cases where algorithm-based processing does not fully resolve curve complexity.

Calculation of the preliminary bread yield

Baking tests were performed for all formulations under standardized conditions. Dough weight, baking losses, and final bread weight were determined experimentally. Dough yield was subsequently calculated using standard technological relationships based on the measured parameters and water absorption determined by the Mixolab system. Optimal dough consistency was achieved by adjusting water addition to reach a target consistency of 1.1 ± 0.05 Nm, as measured by Mixolab.

Bread yield was calculated using the following relationship:

$$\frac{[DW]^{MuE;\,OaE;\,YeE} - [BL]^{MuE;\,OaE;\,Yee}}{[DW]^{WF} - [BL]^{WF}} = \text{Bread Yield Effect}$$

DW [dough weight, kg]; BL [baking loss, %]

Baking loss (BL) was calculated as follows: BL (%) = [(dough weight − bread weight) / dough weight] × 100

The effect of individual additives on yield (increase/decrease relative to control samples prepared from wheat flour without additives) was subsequently evaluated.

Statistical Analysis

Each composite flour formulation was prepared independently in three separate batches. Each batch was then analysed using Mixolab to determine dough rheological properties under standardized testing conditions. Microsoft Excel (**Microsoft Corporation. Microsoft Excel 365, 2018**) was used to evaluate the obtained data, and the XLSTAT 2020.5.1 program (**Lumivero, New York, 2023**) was used for statistical analysis. Analysis of variance – ANOVA was used for statistical evaluation and Duncan's test was used to determine the evidence of statistically significant differences between individual samples ($P \le 0.05$).

RESULTS AND DISCUSSION

Bread is a staple food consumed worldwide, playing a vital role in nutrition thanks to its balanced composition of macronutrients and provision of essential micronutrients (**Rossel, 2011**). Beyond its nutritional value, bread also carries significant cultural and symbolic meaning, conveying messages related to religion, social class, and well-being. According to the European Survey on Consumer Attitudes to Bread, consumers can be broadly categorized into two groups: frequent buyers who prioritize quality and pleasure, and less frequent buyers who focus on nutritional aspects, shelf life, and energy value (**Lambert et al., 2009**). However, a common thread among both groups is a growing awareness of the link between nutrition and health.

In response to this trend, our research explored the potential of enhancing bread nutrition by incorporating extracts from medicinal mushrooms (Maitake, Shiitake, Cordyceps, and Reishi), oat β-glucan extract, and yeast extract - all rich in polysaccharides - into composite flours for bread production. The biological properties of polysaccharide extracts discussed in the Introduction are not directly addressed in the present experimental study, which focuses on their technological and rheological effects. However, this broader context provides a rationale for their application in cereal systems and helps to interpret their use even in cases where certain technological parameters may be less favourable but remain acceptable in view of their potential benefits.

A key factor in bread production is water absorption (WA), a parameter of practical importance to the baking industry. WA is influenced by the individual components of flour, including starch, protein, and fibre. Notably, the damage to starch grains during the grinding process can positively impact water absorption, as observed in classic wheat flour (**Shewry, 2023).** By understanding the factors that affect WA, bakers can optimize their recipes and production processes to create higher-quality bread products.

The addition of extracts from medicinal mushrooms significantly decreased water absorption, despite the fact that a significant proportion of them was β-glucan, which is considered a soluble fibre. Additions of yeast and oat extracts, also containing β-glucan, on the other hand, increased the binding as expected, most notably the addition of oat β-glucan extract. The observed values decreased or increased in direct dependence on the amount of the addition (Figure 1). Despite the fact that all β-glucans have the same type of glycosidic bond connecting glucose molecules, their structural characteristics can vary greatly. These differences include variations in chain length, chain conformation, glycosidic bond positions, degree of branching, branching pattern, three-dimensional structures, both molecular weight and solubility (**Su et al., 2016; Ahmad and Kaleem, 2018; Kaur *et al.,* 2019; Garcia *et al*., 2022**). β-glucans isolated from different sources differ in the reported characteristics, which may be the reason for their different behaviour in composite flours.

It should be noted that detailed physicochemical characterization of the polysaccharide extracts (e.g. molecular weight distribution, solubility, or viscosity contribution) was not performed in this study, as the focus was on their technological functionality in composite flour systems. The extracts used were commercially standardized products with declared composition.

The economic evaluation is based on experimentally obtained processing parameters and reflects standard bakery practice, although detailed quality assessment of final products was beyond the scope of this study. Water absorption (WA) is a key indicator that allows predicting the economy of final product production, in the case of this study, bread. If the binding of wheat flour, which is used as a basis for mixing composite flours, is at the level of 62.3%, it means that 162.3 kg of dough can be prepared from this flour, from which, after baking and after deducting losses during baking (13.7%), 140.2 kg of bread can be formed, which is approximately 280 loaves weighing 500 g each. If we compare these data with the use of composite flour, in which the addition of medicinal mushrooms was 15%, the results will be significantly less economically suitable. The average water absorption of such composite flour is at the level of 46.6% (since, as mentioned above, the addition of medicinal mushrooms reduces the water absorption in proportion to its amount). With such a water absorption, 146.6 kg of dough with the required consistency (1.1 Nm determined on Mixolab) is formed from 100 kg of flour. From this amount of dough, 255 loaves weighing 500g each can be prepared, i.e. 25 loaves fewer for every 100 kg of composite flour compared to the control wheat flour.

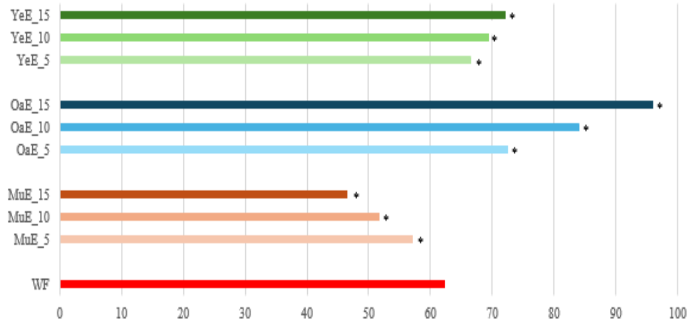

Figure 1 Water absorption (WA) of wheat flour and composite flours enriched with polysaccharide extracts

Legend: WF **[**Wheat flour T650**]**, MuE [Medicinal mushrooms extracts], OaE [Oat β-glucan extract], YeE [Yeast extract], 5/10/15 [amount of addition 5%, 10%, 15%]; Values are presented as mean ± SD (n = 3). Due to the very low variability of the measurements, error bars are smaller than the symbol size and are therefore not displayed. *indicates statistically significant differences ($p < 0.05$) compared to control (WF).

In the case of other additives, the main component of which was β-glucan from oats and yeast, the water absorption increased proportionally with increasing quantities. In the case of β-glucans from oats in an amount of 15% up to 96%, which means that from 196 kg of dough we get 341 loaves weighing 500 g, which is 61 loaves more for every 100 kg of composite flour compared to the control wheat flour. In the case of yeast extract (15% addition), it was finally possible to produce 19 more loaves from 100 kg of such flour for every 100 kg of composite flour compared to control wheat flour. Table 3 shows the technological and economic parameters of dough and bread obtained from composite flour recipes. Dough weight, baking losses and final bread weight were determined experimentally in baking trials carried out under standardized conditions. Water absorption was determined using farinographic measurements. Dough yield was calculated using standard technological relationships based on experimentally obtained parameters. The values represent actual processing data; no predictive modelling was used.

Table 3 Technological and economic parameters of dough and bread obtained from composite flour formulations.

Flour	Dough weight, kg/from 100 kg of flour	Baking loss, %	Bread weight, kg from 100 kg of flour	BYE	Quantity of loaves weighing 0.5 kg
WF	162.5a	13.7a	140.2a	1.00	280.4a
MuE_15	146.5b	13.0b	127.5b	0.91	255.0b
OaE_15	196.0c	12.9b	170.6c	1.22	341.2c
YeE_15	172.2d	13.1b	149.8d	1.07	299.6d

Legend: WF **[**Wheat flour T650**]**, MuE [Medicinal mushrooms extracts], OaE [Oat β-glucan extract], YeE [Yeast extract], BYE [bread yield effect], 15 [amount of addition 15%]; different letters mean significant differences between groups ($p \leq 0.05$)

It is clear from the above that, in addition to nutritional benefits, the addition of suitable polysaccharides also improves the yields of final production (BYE). In this respect, β-glucan from oats and yeast extract can be recommended as suitable from the extracts we have verified. Bieniek and Buksa came to similar conclusions, stating that among non-starch polysaccharides (NSPs), arabinoxylans and β-glucans play the most important role in bread production. Their addition causes an increase in the water absorption in the flour and, as a result, forces the use of a greater addition of water to achieve the desired consistency of the dough. Even a small addition can affect the yield of the dough and allows more bread to be made from the same amount of flour, while such bread is less caloric (**Bieniek and Buksa, 2023**).

However, realistically, determining the water absorption of the flour is only the first step, which allows you to prepare the dough with the desired consistency (500 BJ or 1.1 Nm). Kneading the dough and then changing its physical properties is one of the key steps that determine the mechanical properties of the dough and that have a direct impact on the quality of the final product (**Rosell, 2011; Don, 2022; Wieser *et al.*, 2023**). Farinographic curves or Mixolab curves, record the behaviour of the dough and measure the resistance that the kneading dough puts on the kneading paddles, which provides information about dough development time (DDT), dough stability (DS), degree of softening (SOF) and consistency (**Hadnadjev *et al.*, 2011**).

The addition of polysaccharides increases the fibre content of the flours, which makes it possible to obtain products of both technological and nutritional quality and acceptable to consumers (**Rosell *et al.*, 2010**). However, the high fibre content leads to changes in the rheological properties of the dough, e.g. composite flours with a higher proportion of oats show higher dough stability and a lower degree of softening in farinographic tests (**Veraverbeke and Delcour, 2002; Gautério, 2022; Astiz *et al.*, 2023**). Gluten proteins play a predominant role in determining the properties of dough and the quality of bread production, but other components of flour also affect its functionality and that some may interact with the gluten network and modify its properties (**Wieser *et al.*, 2023; Shewry, 2023**).

The incorporation of extracts in our experiments compared to the control wheat flour T650, the quality of which in terms of rheological properties was only average (dough development 2.2 min, dough stability 2.7 min), significantly influenced macro rheological parameters. Since significant differences were found in these parameters (unlike WA) even between specific extracts of medicinal mushrooms, these are evaluated individually. Compared to wheat control flour, most of the additions extended the DS in particular, which could be considered a very favourable technological impact. In the case of Shiitake, Cordyceps, Reishi extracts and β-glucan from oats, the stability of the dough was extended with the amount of the addition, reaching values 6.8-8.2 times higher than in the control flour. The addition of yeast extract affected the stability of the dough the least significantly, and with additions of 5% and 10%, the stability remained at the same level as with control flour. With Maitake additions, the behaviour of composite flour was different, and after a significant extension of the DS at lower additions, a shortening of the DS was found at the addition of 15% (Figure 2a).

Medicinal mushroom additions did not significantly affect DDT, but β-glucan additions from other sources significantly prolonged DDS, especially oat β-glucan (Figure 3a). Unlike DS, the prolongation of DDT cannot be considered a desirable change, as it makes dough preparation more difficult, both in terms of kneading time and energy consumption. Although an increase in dough development time is generally considered technologically unfavourable, this parameter should be interpreted in the context of overall dough behaviour. In the present study, prolonged DDT was accompanied by a substantial increase in dough stability, indicating improved resistance of the dough system during mixing. From a practical perspective, extended mixing time represents a manageable process adjustment rather than a limiting factor, although it may slightly increase energy demand. Moreover, the applicability of higher extract additions is also driven by their nutritional and functional benefits, which may justify such technological trade-offs. It should also be emphasized that the observed effects are dependent on the type and level of extract addition and should not be generalized across all systems (Figures 2a–4a). The applied interpretative evaluation of Mixolab curves further contributed to a more accurate description of these relationships, particularly in systems exhibiting non-standard rheological behaviour.

During the initial kneading, the wheat dough is subjected to large uni- and biaxial deformations. In addition, the material is distributed at the same time, the originally spherical protein particles are disturbed, and the flour component is hydrated, which, together with the stretching and straightening of the proteins, leads to the formation of a three-dimensional viscoelastic structure with gas retention (**Rosell, 2011**). Wheat flour, when kneaded with water, forms a typical dough peak on the mixolab/farinograph curve (Figure 5a). Today, DDT and/or energy to peak is still an important factor for any researcher or quality assurance manager in wheat technology. However, some composite flours can be rated as very strong because they have not one, but two peaks on the curve **(Don, 2022**). The question is whether the Mixolab® software, which is primarily set for the evaluation of wheat flours, can cope with such a nonstandard course.

An important parameter when evaluating the strength of flour is the degree of softening (SOF), which represents the breakdown of gluten through extended kneading. From Figure 4a, it is possible to read that except for composite flour with the addition of Maitake extract in the amount of 15%, which behaved completely opposite, the other additions reduce the SOF in proportion to the amount of the addition. However, even this evaluation does not fully correspond to a realistic assessment of the rheological properties of composite flours with the addition of polysaccharides, which would be useful for describing the actual dough properties important for kneading dough and ensuring the best technologically appropriate procedures.

In this context, it was necessary to reconsider the evaluation strategy and apply an additional interpretative method (as described in the Materials and Methods section), which may be considered a methodological contribution of the present study. It should be noted that the manual evaluation represents a secondary interpretative approach applied to identical raw curves, rather than an independent experimental measurement.

Manual evaluation of mixolab curves by farinograph practice

We have modified the methodology for evaluating mixolab curves to optimize it and adapt it to the different behaviour of doughs with additives containing different polysaccharides. The curves were visually evaluated and manually calculated, considering the two-peak curve, which was a problem for evaluation by the software. Figure 5a, b, c, d, e, f, g document the waveforms of the curves and their new (recalculated) evaluation, and Figures 2b, 3b and 4b show the adjusted results of DDT, DS and SOF, which more accurately describe the behaviour of doughs with additions. The data provided in the blue frame (Figure 5) were determined by mixolab software, the values marked red, violet and black colour next to (Figure 5) were determined manually by classical farinographic assessment, in which the

DDS is at the point of the upper maximum of the curve, the DS is defined by the points on the upper line of the curve that intersect the maximum consistency given by the centre of the curve, and the degree of softening is measured at the 12th minute after maximum in the middle of the curve. The observed discrepancy between software-based and manual evaluation highlights a limitation of standard Mixolab algorithms in processing non-standard dough systems exhibiting dual-peak torque behaviour. The manual interpretation should therefore be understood as an exploratory refinement aimed at improving interpretability rather than replacing the instrumental output.

The fact that it is possible to prepare a dough with the desired consistency properties from flour and water is conditioned by the presence of gluten proteins, which are stored in the starch endosperm cells of mature grains as separate protein bodies (**Shewry, 2023**). By adding water to the flour and kneading, the gluten matrices in the individual cells combine to form a three-dimensional network with the input of mechanical energy and an increase in the hydration of the gluten protein. The water absorption of wheat flour is related to this moisturizing ability of wheat proteins, and this condition is reflected in the mixolab curve with a peak characterizing the optimal consistency of the dough, normally achieved in 2-3 minutes of kneading (Figure 5a). How long the dough stays in the desired consistency during further mixing is already expressed as the stability of the DS dough, and for strong quality wheat flours, the stability is up to about 10 minutes. The wheat flour used in our experiments was rather weak; its stability was only 2.7 minutes on average.

The predominant role of gluten proteins in determining the properties of dough and the quality of bread production often leads to the fact that other components of flours are neglected. However, other components of the grain affect functionality, and some interact with the gluten network to modify its properties (**Shewry, 2023**). Such a group of ingredients are undoubtedly carbohydrates, which are part of the applied additives - extracts from medicinal mushrooms, oats and yeast extract. As a result of their presence, after the initial hydration, further water binding occurs, thanks to these polysaccharides. The result is a second peak in a row, which confuses the software of the device and no longer evaluates it. The result of the software evaluation is subsequently a very long stability of such doughs, which does not correspond to the real situation, and we evaluate it as incorrect information. The preparation of such doughs until the optimal consistency is reached requires special regimes of kneading. We assume that the dough development lasts until the second peak is formed and only then will the dough be formed, which has hydrated all the water it could, has the right consistency and will give us a quality product.

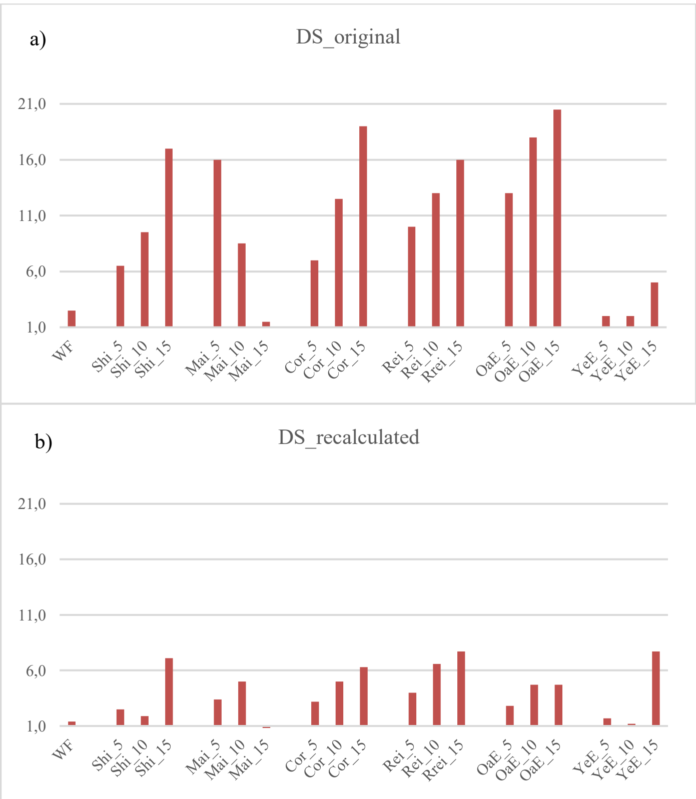

Figure 2 Dough stability evaluated a) by Mixolab software; b) manually by farinograph practice

Legend: WF [Wheat flour T650], Shi [Shiitake extract], Mai [maitake extract], Cor [Cordyceps extract], Rei [Reishi extract], OaE [Oat β-glucan extract], YeE [Yeast extract], 5/10/15 [amount of addition 5%, 10%, 15%] Values a) are presented as mean ± SD (n = 3). Due to the very low variability of the measurements, error bars are smaller than the symbol size and are therefore not displayed.

As can be seen from the figures characterizing the stability of the dough (Figure 2a), it was originally possible to read (based on the evaluation by the instrument) that the additions of Shiitake extract, Cordyceps extract, Reishi extract, and oat β-glucan extract very significantly prolong the stability of the dough. However, after recalculation, the situation is less favourable, although it can still be stated that the additives extend dough stability, except for three samples that also behaved differently when evaluating dough development time: Maitake extract 15%, yeast extract 5% and yeast extract 10% (Figure 2b). In the case of Shiitake extract, the increase in stability was interesting only at the amount of the addition of 15%, in the case of others, with the already mentioned exceptions, the stability was extended with increasing the amount of the addition, namely 3.4 – 5.5 times compared to wheat control flour. However, the DS did not exceed the limit of 8 minutes in any of the samples, although in the original evaluation it was intended for more than 20 minutes.

Based on the evaluations by the software of the device, conclusions were reached that, except for the addition of beta-glucan from oats and, to a lesser extent, yeast extract, the selected additives did not cause a significant change in the dough development time (Figure 3a). However, after recalculation based on the classical methodology for the evaluation of farinographic curves (Brabender® Farinograf), the conclusions are different. All additions caused a significant extension of the dough development time, mainly in relation to the amount of the addition (Figure 3b). The only exception was the addition of maitake extract at the highest selected amount (15%), which resulted in a very irregular curve profile that did not allow reliable extraction of the standard Mixolab parameters (Figure 5c). This type of behaviour reflects the structural instability of the dough. The addition of yeast extract in the amount of 5% and 10% did not change the development of the dough.

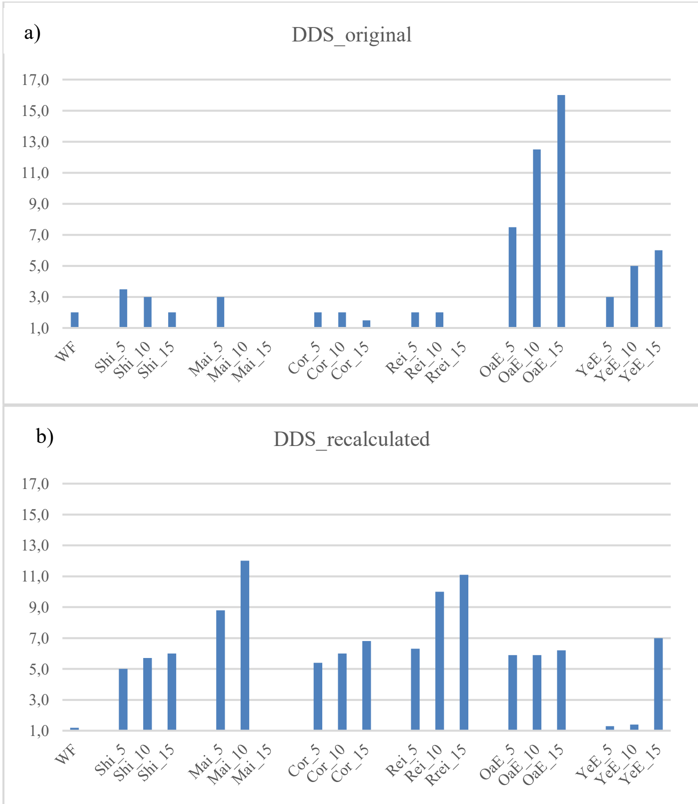

Figure 3 Dough development time evaluated a) by Mixolab software; b) manually by farinograph practice

Legend: WF [Wheat flour T650], Shi [Shiitake extract], Mai [maitake extract], Cor [Cordyceps extract], Rei [Reishi extract], OaE [Oat β-glucan extract], YeE [Yeast extract], 5/10/15 [amount of addition 5%, 10%, 15%] Values a) are presented as mean ± SD (n = 3). Due to the very low variability of the measurements, error bars are smaller than the symbol size and are therefore not displayed.

The discrepancy between software-based and manual evaluation highlights a limitation of standardized algorithmic processing in complex composite flour systems, suggesting the need for complementary interpretative approaches. Based on manual evaluation, we conclude that extracts additions generally increase degree of softening (Figure 4b), in contrast to findings through mixolab software (Figure 4a). Compared to the control, the values were lower only for Mai_10, Rei_10 and Rei_15, but as shown in Figure 5c and 5e, the waveforms of the curves were non-standard. The substitution of wheat flour with non-baker raw materials must be approached with the required attention and individually according to the specific ingredient and its origin in terms of dough processability.

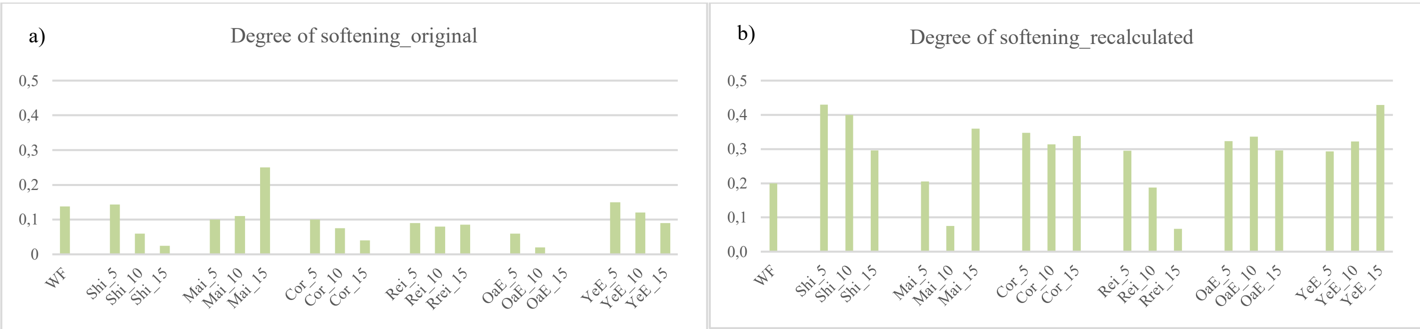

Figure 4 Degree of softening evaluated a) by Mixolab software; b) manually by farinograph practice

Legend: WF [Wheat flour T650], Shi [Shiitake extract], Mai [maitake extract], Cor [Cordyceps extract], Rei [Reishi extract], OaE [Oat β-glucan extract], YeE [Yeast extract], 5/10/15 [amount of addition 5%, 10%, 15%] Values a) are presented as mean ± SD (n = 3). Due to the very low variability of the measurements, error bars are smaller than the symbol size and are therefore not displayed.

The waveforms of the curves measured on the Mixolab and the methods of their evaluation are documented in a series of Figures 5, which we consider necessary to include completely, for a better understanding of the measured differences. It is necessary to read macrorheological phenomena with an understanding of the processes that take place in the kneading dough. Given that in current cereal research there is a relatively large attention paid to the addition of non-bakery raw materials with different levels of processing, it is necessary to adjust the evaluation in the case of non-standard mixolab or farinograph curves.

Our research and the modified method of evaluating rheological properties of mixed dough that follows from it, offers a possibility to more accurately assess the processing of bakery semi-finished products and set ideal technological procedures.

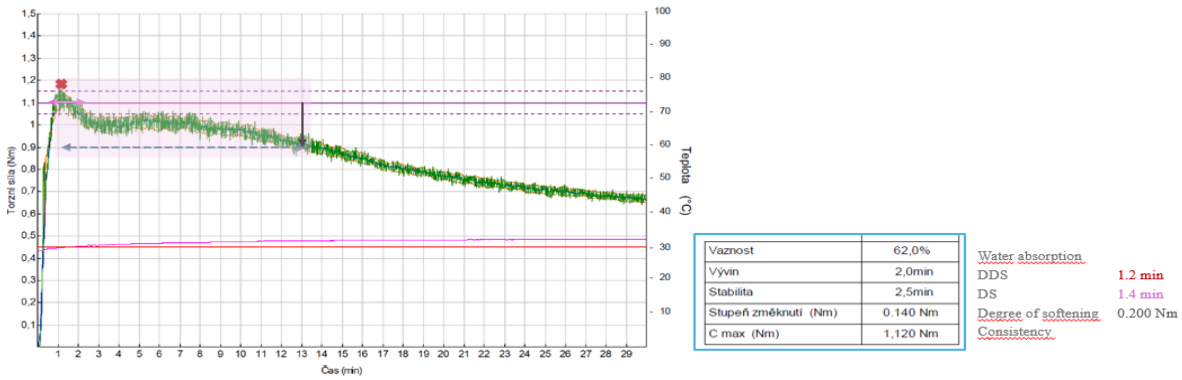

Figure 5a Mixolab curve of wheat flour with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

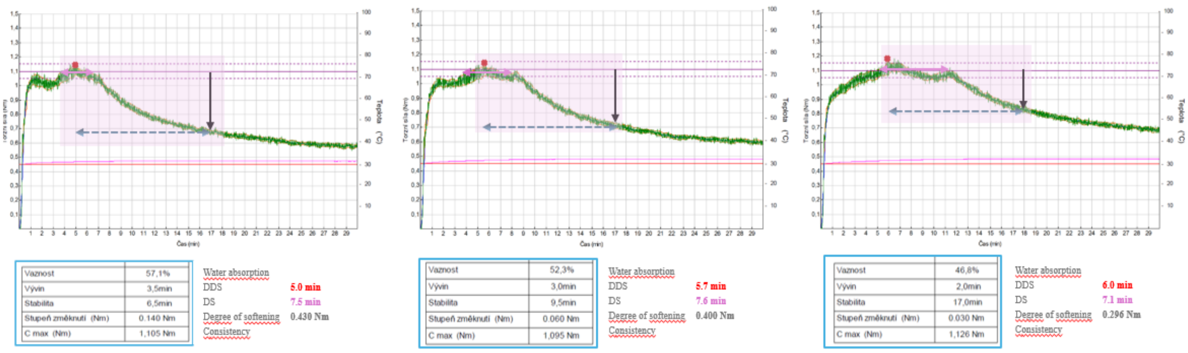

Figure 5b Mixolab curve of composite flours with shiitake extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

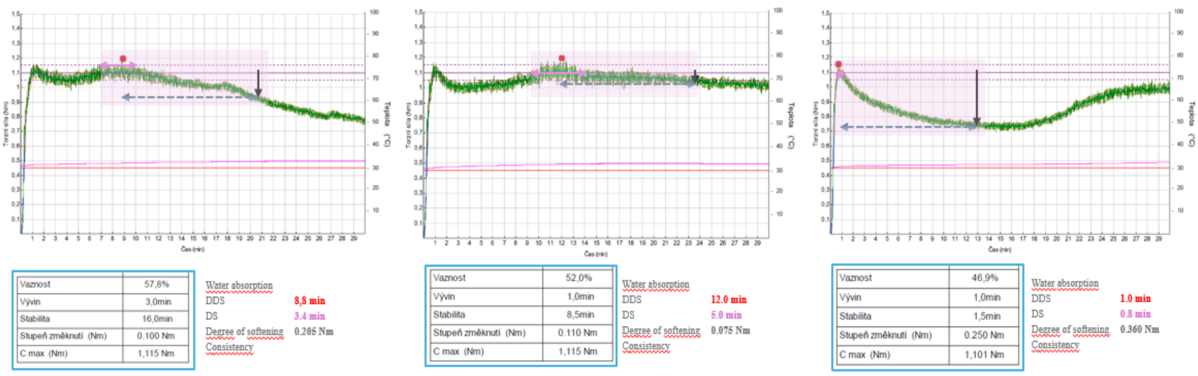

Figure 5c Mixolab curve of composite flours with maitake extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

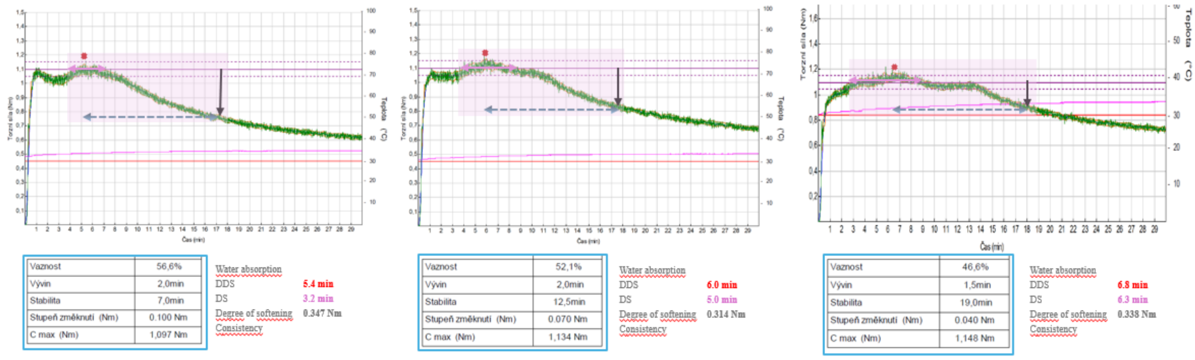

Figure 5d Mixolab curve of composite flours with cordyceps extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

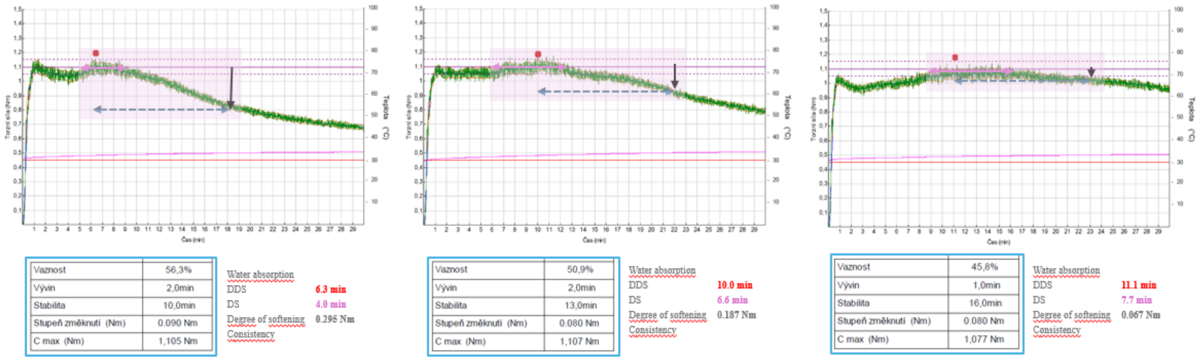

Figure 5e Mixolab curve of composite flours with reishi extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

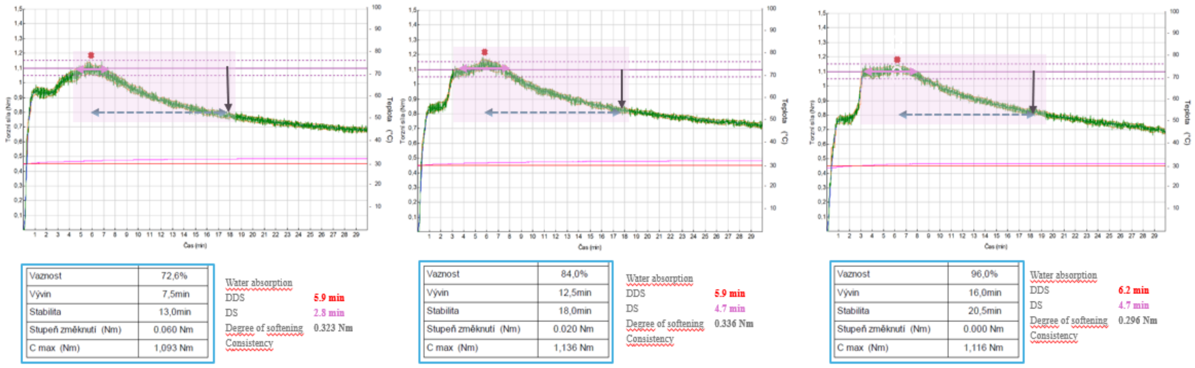

Figure 5f Mixolab curve of composite flours with oat β-glucan extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

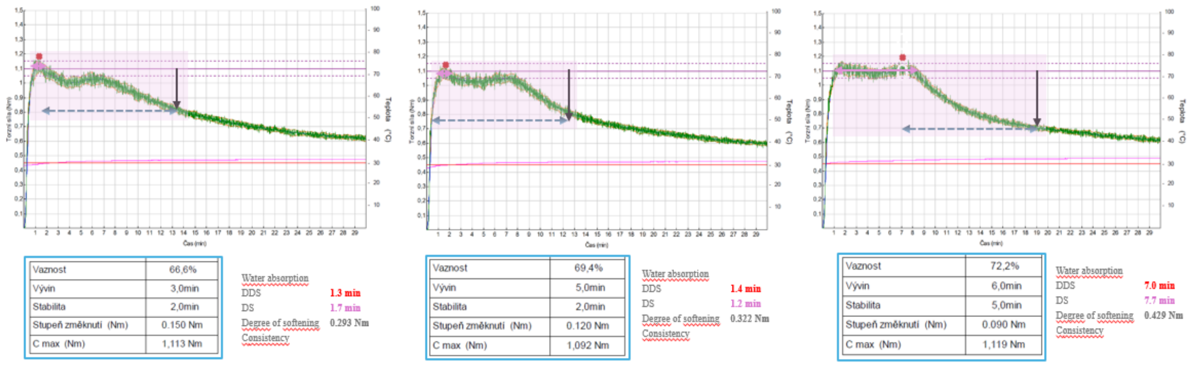

Figure 5g Mixolab curve of composite flours with yeast extract with marked values by Mixolab software (blue frame) and manually by farinograph practical (DDS red colour; DS violet colour; Degree of softening black colour)

It should be noted that the present study focuses on the rheological behaviour of dough systems and their technological implications. These parameters are widely used as indicators of processing performance in cereal science; however, they do not fully represent final bread quality. The results should therefore be understood within the context of dough rheology and technological evaluation.

CONCLUSION

As part of the research, the effects of adding medicinal mushroom extracts, oat β-glucan extract, and yeast extract - all rich in polysaccharides - to composite flours for bread production were investigated. The rheological behaviour of the resulting dough was assessed using Mixolab measurements, with a focus on key parameters such as dough development time (DDT), dough stability (DS), and degree of

softening (SOF). The evaluated parameters expressed by the Mixolab software were supplemented by manual evaluation using the conventional Farinograph-Brabender method. The parameters generated by the Mixolab software were supplemented by manual evaluation using the conventional Farinograph-Brabender method. Given the irregular course of the Mixolab curves, manual assessment serves as an additional interpretive framework that facilitates a better description of anomalous behaviour, specifically accounting for individual variations and the occurrence of double peaks.
Manual evaluation revealed that the dough development time (DDS) in composite flours was generally significantly longer than in the control flour, with one notable exception: the addition of yeast extract in quantities up to 10%. This prolonged DDS has important implications for the kneading process, as it requires increased time and energy consumption to achieve optimal results. This finding highlights the need for careful consideration of the kneading process when working with composite flours containing these types of additives.
The addition of extracts had a positive impact on dough stability, resulting in an optimal consistency that lasted longer. This was evident in both the Mixolab software analysis, which showed a stability increase of 6.8-8.2 minutes compared to the control dough, and manual evaluation, which revealed a stability increase of 3.4-5.5 times. These findings suggest that, particularly when working with non-standard composite flours, manual curve reading using the Farinograph-Brabender method is the preferred approach.
In addition to dough stability, another crucial quality parameter - water absorption (WA) - was also investigated. The results showed that the addition of medicinal mushroom extracts reduced WA values by up to 25%, depending on the amount added, whereas oat β-glucan extract and yeast extract increased WA by up to 16% and 54%, respectively. Notably, these composite flours offer economic benefits in the production of final products, as they enable the preparation of 19-61 more 500g loaves per 100kg of flour compared to the control flour.
From a rheological perspective, the data indicate that additives containing polysaccharides, such as medicinal mushroom extracts (Shiitake, Cordyceps, Reishi), oat β-glucan extract, and yeast extract, can be recommended at levels up to 15% (or 10% for Maitake extract). These additives enhance the processability of doughs, making them a valuable option for improving the overall quality and efficiency of baked goods production.
This study provides both a systematic evaluation of the rheological behaviour of composite flours enriched with polysaccharide-rich extracts and a methodological contribution in the interpretation of Mixolab curves under non-standard conditions. The combination of these approaches allows for a more comprehensive understanding of complex dough systems and improves the reliability of technological assessment in such formulations.

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