# Enzyme technology for oat milk production

*Hristina* Panajotova[1*], *Valentina* Dobreva[2], *Boriana* Zhekova[1] and *Georgi* Dobrev[1,3]

[1]Department of Biochemistry and Nutrition, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria
[2]Department of Engineering Ecology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria
[3]Center of Competence AgriFoodSystems and Bioeconomy, 4000 Plovdiv, Bulgaria

**Abstract.** Oat milk production through enzymatic hydrolysis was investigated. The enzymatic hydrolysis involved liquefaction with α-amylase at 70 °C, and subsequent sacharification with glucoamylase or maltogenic amylase at 60 °C. The chemical composition of different oat flour fractions was studied and oat flour fraction with fine particles size (75-150 μm) was selected for oat milk production. The effect of hydromodule on chemical composition, dry matter yield and rheological behaviour of oat milk was investigated. The optimal hydromodule of the oat slurry was determined to be 1:6. At hydromodule 1:6 the largest relative viscosity decrease ($\Delta\eta \approx 90\%$), high dry matter yield (61-65 %) and homogeneous liquefaction of the slurry was achieved. Both investigated enzymes (glucoamylase and maltogenic amylase) were considered suitable for efficient hydrolysis of oat slurry. Based on the reducing sugars released, the most favorable conditions for enzyme hydrolysis were identified to be 0.10% glucoamylase for 60 min at 70 °C and 0.10% maltogenic amylase for 60 min at 60 °C. Under these conditions, oat milk, with balanced macronutrient composition (protein = 0.35-0.46 %, lipids = 0.47-0.48 %), high sugar content (6.6-14.2 mg/ml), and favorable rheological properties was produced.

## 1 Introduction

The growing demand for plant-based alternatives to dairy products has stimulated considerable research interest in cereal-derived beverages such as oat milk. Oats are rich in proteins, β-glucans, and complex carbohydrates that provide nutritional and functional advantages, including cholesterol-lowering and prebiotic effects [1]. However, the high starch and fiber content of oat flour leads to elevated viscosity and poor filterability, which complicate processing and limit product stability.

Enzymatic hydrolysis represents an efficient and sustainable approach to overcome these challenges [2-5]. The enzymatic hydrolysis usually involves liquefaction and saccharification with starch degrading enzymes [6].

α-Amylase is predominantly used for liquefaction [7], as the enzyme catalyzes endo-hydrolysis of internal α-1,4-glycosidic linkages in starch granules α-Amylase acts randomly within the polysaccharide chain, resulting in a rapid viscosity reduction and generation of soluble dextrins.

The saccharification step is usually performed with glucoamylases or maltogenic amylases. Glucoamylase hydrolyzes both α-1,4- and α-1,6-glycosidic bonds, releasing glucose as the main product, resulting in rapid accumulation in reducing sugars [5]. Maltogenic amylase primarily cleaves α-1,4-linkages from the non-reducing ends of dextrins, producing maltose and oligosaccharides [8].

The enzymes used for starch hydrolysis are usually heat stable at temperatures of 60-70°C, allowing simultaneous complete gelatinization and molecular dispersion of starch.

The combined use of liquefying α-amylase and saccharifying enzyme allows controlled degradation of starch and dextrins, reducing viscosity, and generating maltose and glucose that enhance the sweetness and sensory profile of the final beverage [7]. Compared with mechanical or thermal treatments, enzymatic liquefaction operates under milder conditions, preserves heat-sensitive nutrients, and improves the functional properties of plant-based matrices.

The enzymatic hydrolysis of oats to produce oat milk depends on multiple parameters such as particle size, hydromodule ratio, temperature, enzyme concentration, and hydrolysis time.

Particle size is crucial for enzyme action, as it affects the specific surface area of the substrate, enzyme accessibility, and water availability [9].

The hydromodule ratio is important for enzyme hydrolysis, by not only affecting the substrate concentration, but also providing homogeneous liquefaction, sufficient enzyme diffusion and optimal viscosity of the final product.

The temperature of the enzymatic process is important for efficient degree of hydrolysis and it ensures optimal starch gelatinization.

Factors like enzyme concentrations and hydrolysis time are necessary to be investigated for every specific enzyme.

* Corresponding author: angelovatina84@yahoo.com

Despite the increasing industrial application of enzymatic oat processing, systematic evaluation of the relationships among these process parameters remains limited.
Therefore, the objective of this study is to investigate the enzymatic hydrolysis of oat slurry for oat milk production and to determine the influence of particle size, hydromodule ratio, and enzymatic treatment on the rheological and compositional properties of oat milk.

## 2 Materials and methods

### 2.1 Milling and fractioning of oat grains for oat milk production.

Oat grain was milled and the row material was fractioned by using sieves with different hole sizes. Three oat flour fractions with particle size, varying in the range of 75–150 μm, 150–250 μm, and above 250 μm were prepared. The whole oat grains and the separate fractions were analysed for moisture content, water holding capacity (WHC), oil holding capacity (OHC), starch, fiber, pentosanes, protein, and lipids content.

### 2.2 Enzyme hydrolysis of oat slurry

Out slurries with hydromodule 1:4, 1:6 and 1:7 (w/v) were prepared by mixing the respective quantity of the row material with water. Enzyme hydrolysis of oat slurry was performed with different enzyme preparations supplied by DSM Food Specialties (Delft, Netherlands): Delvo®Plant ALT (α-amylase), Delvo®Plant GLU (glucoamylase), and Delvo®Plant MAL (maltogenic amylase).
ALT was applied for the liquefaction step at 70 °C under continuous stirring at 100 rpm to ensure complete gelatinization and molecular dispersion of starch.
Following liquefaction, two alternative saccharification enzymes were tested separately (GLU and MAL). GLU was applied at 70 °C, and MAL was applied at 60 °C.
Aliquots were withdrawn at each time point, immediately heated at 95 °C for 5 min to inactivate the enzyme, cooled to room temperature, and analyzed for the corresponding parameter (apparent viscosity, reducing sugars, protein content, and lipids content).
The influence of enzyme concentration and hydrolysis time on reducing sugars content was studied at hydromodule 1:6 (w/v) with enzyme dose of GLU or MAL in the range of 0.10-0.40% for 30-90 min.

### 2.3 Measurement of viscosity of oat slurry

The apparent viscosity of oat slurries before and after enzymatic treatment was measured by using a controlled-stress rheometer equipped with a cone-and-plate geometry (e.g., 40 mm, 2°) and Peltier temperature control. Samples were pre-sheared at 100 $s^{-1}$ for 30 s and allowed to rest for 60 s prior to measurement. Shear rate was increased logarithmically from 10 to 100 $s^{-1}$ (10 points per decade; 5 s per point). Apparent viscosity was calculated as $\eta = \tau/\gamma$ at each point. Measurements were conducted at 30 °C, 50 °C, and 70 °C. Evaporation was minimized using a solvent trap. The relative decrease in viscosity ($\Delta\eta$, %) was calculated according to the following equation:

$$\Delta\eta(\%)=\eta_0-\eta_t/\ \eta_0 \times 100 \qquad (6)$$

where $\eta_0$ is the viscosity of the untreated slurry and $\eta_t$ is the viscosity after enzymatic treatment at time t.

### 2.4 Determination of total and refractometric dry solids

The total solids (TS) content of the oat slurries before enzymatic hydrolysis was determined gravimetrically by drying at 105 °C to constant weight. Approximately 5.0 g of homogenized sample were placed in pre-dried, pre-weighed aluminum dishes and dried in a thermostatic oven (Memmert, Germany) until no further weight loss was observed. TS was calculated as the percentage of dry residue relative to the initial sample mass (%, w/w).
After enzymatic hydrolysis, the refractometric dry solids (RDS) of the clarified supernatant were measured using a digital refractometer (temperature-controlled at 70 °C). Samples were first centrifuged at 4000 × g for 10 min, and the clear fraction was used for measurment.

### 2.5 Determination of dry matter yield

The yield of oat milk after enzymatic hydrolysis was evaluated in terms of dry matter yield (YDM), calculated from the total solids content of the initial slurry and the filtrate obtained after separation of the insoluble residue:

$$YDM(\%)= Vm\times TSm/\ V_0\times TS_0\times 100 \qquad (7)$$

where:
$V_0$ - initial volume of the oat slurry (ml),
$TS_0$ - total solids content of the slurry before hydrolysis (%),
Vm - volume of the filtrate (ml) after enzymatic hydrolysis,
TSm - total solids content of the oat milk after hydrolysis (%).

### 2.6 Assays

All assays were performed in triplicate and data are expressed as mean value ± standard deviation.

#### 2.6.1 Starch determination

Starch content was determined by the acid hydrolysis polarimetric method, adapted from the Ewers procedure [10]. Results were expressed as percentage of starch on a dry-weight basis.

### 2.6.2 Determination of pentosans

Pentosan content was determined colorimetrically using the phloroglucinol–acetic acid method, adapted from the procedure of Douglas [11].

### 2.6.3 Determination of dietary fiber

Total dietary fiber (TDF) was determined using the Total Dietary Fiber Assay Kit (K-TDFR, Megazyme, Ireland), which involved enzymatic digestion with heat-stable α-amylase, protease, and amyloglucosidase, followed by gravimetric quantification of insoluble and soluble fiber fractions, in accordance with AOAC Official Method 991.43 [12].

### 2.6.4 Determination of water-holding and oil-holding capacities

Water-holding capacity (WHC) and oil-holding capacity (OHC) were determined according to the method of Sosulski [13].

### 2.6.5 Determination of reducing sugars

Reducing sugars were determined by the Schoorl method [14,15]. Glucose was used as a standard.

### 2.6.6 Determination of protein content

Protein content was determined by the Kjeldahl method [16,17]. Results were expressed on a dry matter basis.

### 2.6.7 Determination of lipid content

Lipid content was determined by gravimetric solvent extraction with petroleum ether (AOAC, 2019) [18]. The lipid content was expressed as a percentage of total dry matter.

## 3 Results and discussion

### 3.1 Chemical composition and functional properties of oat flour fractions

The chemical composition of the whole oat grains and its sieved flour fractions are summarized in Table 1.
The whole grain contained 55.3 % starch, 16.3 % protein, and 7.2 % lipids. These values were consistent with previously reported compositions of oat grain [19]. Fractionation produced distinct compositional differences among the particle-size classes. The fine fraction (75–150 μm) was notably enriched in starch (66.2 %) and depleted in fiber (7.8 %) and pentosans (5.5 %). The coarse fraction (≥250 μm) exhibited lower starch content (44.3%), but higher levels of fiber (10.4 %) and pentosans (7.8 %).
These trends are in agreement with previous findings showing that finer particles originate predominantly from endosperm material rich in digestible carbohydrates, while coarser particles retain a greater proportion of bran and cell-wall polysaccharides [20].

**Table 1.** Chemical composition and functional properties of whole oat grain and oat flour fractions

| Sample | Moisture (%) | WHC ($gH_2O/g$) | OHC (g oil/g) | Starch (%) | Fiber (%) | Pentosans (%) | Protein (%) | Lipids (%) |
|---|---|---|---|---|---|---|---|---|
| Whole oat grain | 9.8 | 1.5 | 1.7 | 55.3 | 9.8 | 7.1 | 16.3 | 7.2 |
| ≥250 μm | 10.6 | 1.9 | 2.1 | 44 | 10.4 | 7.8 | 13 | 5.3 |
| 150–250 μm | 10.7 | 1.6 | 1.9 | 54 | 9.7 | 6.3 | 13 | 5.1 |
| 75–150 μm | 10.8 | 1.3 | 1.6 | 66.2 | 7.8 | 5.5 | 13 | 4.9 |

All values are mean values of triplicate measurements. The standard deviations were ≤0.3 for WHC, OHC, ≤1.0 for moisture, fibers, and protein; ≤0.7 for pentosans and lipids, and ≤3.0 for starch.

The differences in the chemical compositions of oat flour fractions resulted in different functional properties, regarding the ability for water and oil holding.
The coarse fraction exhibited the highest WHC (1.9 g $H_2O$/g) and OHC (2.1 g oil/g), which was due to the greater content of insoluble fibers and pentosanes.
In contrast, the fine fraction showed the lowest WHC (1.3 g $H_2O$ /g) and OHC (1.6 g oil/g), consistent with its smoother surface and low content of fibers and pentosanes. The fine fraction contained the highest amount of starch (66.2 %), which was inversely correlated with WHC and fiber content. These findings indicated that particle refinement reduced hydration sites, but enhanced starch availability. This effect is expected to influence slurry viscosity and the efficiency of subsequent enzymatic hydrolysis [9, 21]. The fine fraction, characterized by higher starch and lower fiber content, provides a more homogeneous dispersion and facilitates substrate accessibility to enzymes.
Based on these characteristics, the fine oat flour fraction (75–150 μm) was selected for subsequent viscosity and hydrolysis experiments, as it offers an optimal balance between substrate availability and hydration stability.

### 3.2 Evaluation of viscosity profile and flow parameters of oat slurry

The rheological behaviour of oat slurries is a crucial factor of their processability and the sensory characteristics of the resulting oat milk.

Viscosity strongly depends on both the solid-to-liquid ratio and the extent of starch hydrolysis. For these reasons, the combined effects of hydromodule, and enzymatic treatment were studied. The measurement of oat slurry viscosity was performed at different temperatures (30, 50, and 70 °C) and shear rates (20 rpm, 50 rpm and 100 rpm), as the relation of viscosity on temperature and shear rate determine the rheological properties of liquids [22]. The results are presented in Tables 2a–2c.

**Table 2a.** Apparent viscosity of oat slurries with and without enzyme hydrolysis at hydromodule 1:4 at (mean SD = ± 0.1, n = 3)

| Hydromodule (w/v) | T (°C) | η (Pa·s) without enzyme | η (Pa·s) with enzyme | Δη (%) |
|---|---|---|---|---|
| 1:4 | 30 | 20 rpm: 8.8<br>50 rpm: 7.8<br>100 rpm: 6.5 | 20 rpm: 1.5<br>50 rpm: 1.2<br>100 rpm: 1.1 | 82.95<br>84.62<br>84.72 |
| | 50 | 20 rpm: 7.0<br>50 rpm: 5.5<br>100 rpm: 4.7 | 20 rpm: 1.1<br>50 rpm: 0.9<br>00 rpm: 0.9 | 84.29<br>83.64<br>80.85 |
| | 70 | 20 rpm: 3.5<br>50 rpm: 2.5<br>100 rpm: 2.1 | 20 rpm: 1.0<br>50 rpm: 0.9<br>100 rpm: 0.9 | 71.43<br>64.00<br>57.14 |

*Conditions: enzyme dose = 0.10 % (w/w to dry matter); 100 rpm; enzyme hydrolysis with ALT at 70°C and GLU/MAL at 60 °C

At hydromodule 1:4, the slurry showed the highest viscosity (8.8 Pa·s) at 20 rpm and 30 °C, reflecting strong particle interactions and limited water availability. Enzymatic hydrolysis further decreased viscosity (0.9-1.5 Pa·s), yet the relative decrease of viscosity remained high, indicating diffusion limitations within the dense matrix that hindered complete liquefaction. Increasing shear rate and temperature caused decrease in the viscosity, typical of pseudoplastic liquids [22].

At hydromodule 1:6, viscosity values were lower, the highest measured value was 3.0 Pa·s at 20 rpm and 30 °C (Table 2b). The trend for decrease in viscosity with the increase in temperature and shear rate was confirmed, indicating pseudoplastic behavior of the liquid.

Enzymatic treatment led to reduction of Δη to about 90 %, suggesting optimal enzyme–substrate interaction and balanced water availability at hydromodule 1:6. The system was homogenous and maintained uniform flow indicating hydromodue 1;6 to be an effective ratio for enzymatic liquefaction of oat slurries.

Further dilution (hydromodule 1:7) lowered the initial viscosity below 0.8 Pa·s. (Table 2c). The pattern for pseudoplastic liquid characteristics of the slurry was kept. The relative reduction of viscosity (Δη) after enzyme treatment was in the range of 25-50 %.

**Table 2b.** Apparent viscosity of oat slurries with and without enzyme hydrolysis at hydromodule 1:6 (mean SD = ± 0.1, n = 3)

| Hydromodule (w/v) | T (°C) | η (Pa·s) without enzyme | η (Pa·s) with enzyme | Δη (%) |
|---|---|---|---|---|
| 1:6 | 30 | 20 rpm: 3.0<br>50 rpm: 2.6<br>100 rpm: 2.2 | 20 rpm: 0.7<br>50 rpm: 0.6<br>100 rpm: 0.5 | 76.67<br>76.92<br>77.27 |
| | 50 | 20 rpm: 2.1<br>50 rpm: 1.8<br>100 rpm: 1.6 | 20 rpm: 0.3<br>50 rpm: 0.2<br>100 rpm: 0.2 | 85.71<br>88.89<br>87.50 |
| | 70 | 20 rpm: 2.5<br>50 rpm: 2.3<br>100 rpm: 2.1 | 20 rpm: 0.3<br>50 rpm: 0.2<br>100 rpm: 0.2 | 88.00<br>91.30<br>90.48 |

*Conditions: enzyme dose = 0.10 % (w/w to dry matter); 100 rpm; enzyme hydrolysis with ALT at 70°C and GLU/MAL at 60 °C

Additionally, it was considered that the high dilution of the final product might negatively affect the mouthfeel and dry matter recovery of the final oat milk. Thus, higher hydromodules improve processability but compromise the sensory density of the beverage.

**Table 2c.** Apparent viscosity of oat slurries with and without enzyme at hydromodule 1:7 (mean SD = ± 0.1 ,, n = 3)

| Hydromodule (w/v) | T (°C) | η (Pa·s) without enzyme | η (Pa·s) with enzyme | Δη (%) |
|---|---|---|---|---|
| 1:7 | 30 | 20 rpm: 0.8<br>50 rpm: 0.6<br>100 rpm: 0.5 | 20 rpm: 0.4<br>50 rpm: 0.3<br>100 rpm: 0.3 | 50<br>50<br>40 |
| | 50 | 20 rpm: 0.6<br>50 rpm: 0.4<br>100 rpm: 0.2 | 20 rpm: 0.3<br>50 rpm: 0.3<br>100 rpm: 0.2 | 50<br>25<br>0.0 |
| | 70 | 20 rpm: 0.4<br>50 rpm: 0.2<br>100 rpm: 0.1 | 20 rpm: 0.3<br>50 rpm: 0.2<br>100 rpm: 0.2 | 25<br>0.0<br>0.0 |

*Conditions: enzyme dose = 0.10 % (w/w to dry matter); 100 rpm; enzyme hydrolysis with ALT at 70°C and GLU/MAL at 60 °C

The comparative analysis of oat slurries at hydromodules 1:4, 1:6, and 1:7 revealed that viscosity reduction during enzymatic liquefaction depended primarily on solids concentration. Denser systems (hydromodule 1:4) hindered optimal enzyme hydrolysis, the achieved maximum relative viscosity reduction Δη was about 85 %. In contrast, the most diluted slurries (1:7) displayed low baseline viscosity and easy flow, but the enzymatic effect was less pronounced (Δη=25-50 %) probably due to the reduced substrate content.

The intermediate ratio (1:6) achieved the best balance between solid loading and enzyme performance,

resulting in the largest relative viscosity decrease (Δη ≈ 90%) and homogeneous liquefaction. This condition ensured sufficient enzyme diffusion and effective substrate hydrolysis.
Therefore, hydromodule 1:6 was identified as the optimal operational condition for subsequent experiments on enzyme hydrolysis.

### 3.3 Effect of enzymatic hydrolysis on oat milk solids and yield

Enzymatic hydrolysis influence not only the viscosity of the slurry, but it is a main factor determining the concentration of solids in oat milk and the resultant yield of the product.
In Table 3 the results for the effect of enzymatic hydrolysis on oat milk solids and oat milk yield at different hydromodules are presented.

**Table 3.** Effect of enzymatic hydrolysis on total solids (TS), refractometric dry solids (RDS), and dry matter yield at different hydromodules.

| Hydromodule (w/v) | TS before hydrolysis (%) | RDS after hydrolysis (%) | | YDM % | |
|---|---|---|---|---|---|
| | | Glu | Mal | Glu | Mal |
| 1:4 | 21.4 | 15.2 | 14.3 | 66.7 | 77.9 |
| 1:6 | 16.0 | 14.0 | 12.8 | 61.2 | 65.3 |
| 1:7 | 13.1 | 9.5 | 8.5 | 47.1 | 50.3 |

Enzyme dose = 0.10 % (w/w to dry matter); hydrolysis temperature: ALT 70 °C, GLU/MAL 60 °C.

The total solids (TS) before hydrolysis represent the overall initial dry matter, including insoluble starch granules and dietary fibers. The refractometric dry solids (RDS) measured after hydrolysis reflect mainly the soluble carbohydrate fraction after enzyme hydrolysis.
The comparison between TS (before hydrolysis) and RDS (after hydrolysis) highlighted the mass balance and solubilization efficiency of the process. For hydromodule 1:4 the solubilized solids after enzyme hydrolysis represented about 72 % of initial dry solids. With the increase of the dilution to hydromodule 1:6, the solubilized dry matter was increased to about 87 %. Further increase of the water content led to decrease of soluble dry solids to 72 %, probably due to the decrease in substrate concentration in the diluted medium.
The calculated yield showed the highest value at hydromodule 1:4, and it decreased with the dilution of the slurry. At hydromodule 1:7 it decreased to about 47–50 %, depending on the enzyme applied. Although higher dilution (1:7) increased the volume of filtrate, the total solids recovered in the liquid phase were lower, probably due to insufficient substrate amount for optimal enzyme action.

Thus, a hydromodule of 1:6 can be identified as the optimal condition for oat milk production, ensuring maximal solubilization of dry solids at high yield after enzyme hydrolysis.

### 3.4 Effect of enzymatic hydrolysis on the chemical composition of oat milk

#### 3.4.1 Influence of enzyme concentration and hydrolysis time on reducing sugar content

The enzyme hydrolysis of oat slurry was performed at constant 0.10 % (w/w) dose of thermostable α-amylase (ALT) as a liquefying enzyme, followed by a saccharification process with glucoamylase (GLU) or maltogenic amylase (MAL). The effect of enzyme dose of GLU and MAL, as well as the effect of the duration of the treatment on the enzyme hydrolysis were studied (Fig. 1). The enzyme hydrolysis was evaluated by the reducing sugars content of oat milk.

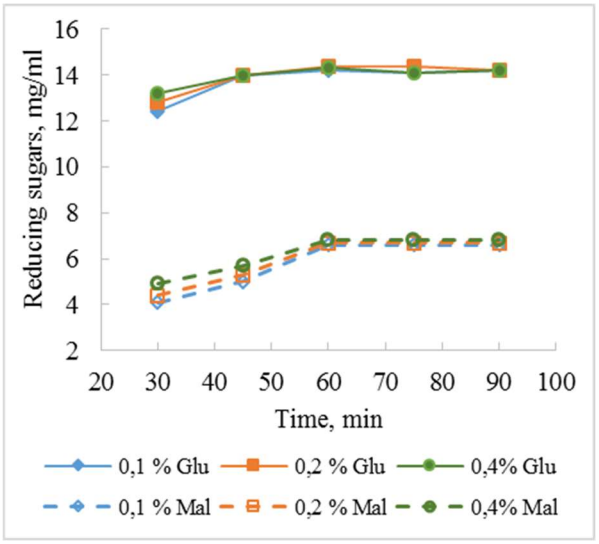


**Fig. 1.** Time- and dose-dependent formation of reducing sugars by GLU and MAL during enzymatic hydrolysis for oat milk production (hydromodule1:6, 60–70 °C).

The results for the enzyme hydrolysis showed that both enzymes efficiently hydrolyzed the substrate. When GLU was used for sacharification, reducing sugars increased from 13.2 mg/ml at 30 min to 14.2 mg/ml at 90 min at enzyme dose of 0.1 %. Higher enzyme levels (0.20–0.40%) resulted in no substantial improvement of hydrolysis process, indicating substrate saturation and possible product inhibition of the enzyme. In contrast, MAL formed lower reducing sugars content, increasing from 4.9 mg/ml to 6.8 mg/ml over the same period. Overall, GLU generated nearly twice the amount of reducing sugars compared to MAL.
Nevertheless, both investigated enzymes were considered suitable for efficient hydrolysis of oat slurry for oat milk production. Based on the reducing sugars content, the most favorable operational conditions were identified to be 0.10 % GLU for 60 min at 70 °C and 0.10 % MAL for 60 min at 60 °C.

### 3.4.2 Chemical composition of oat milk

The compositional analysis of oat milk obtained at different hydromodules (1:4, 1:6, and 1:7) after enzyme hydrolysis with GLU and MAL is presented in Table 4.

**Table 4**. Effect of hydromodule on the composition of oat milk after enzymatic hydrolysis

| Hydromodule (w/v) | Proteins (%) | | Lipids (%) | | Reducing sugars (mg/ml) | |
|---|---|---|---|---|---|---|
| | Glu | Mal | Glu | Mal | Glu | Mal |
| 1:4 | 0.60 | 0.49 | 0.53 | 0.51 | 14.5 | 6.9 |
| 1:6 | 0.46 | 0.35 | 0.48 | 0.47 | 14.2 | 6.6 |
| 1:7 | 0.37 | 0.30 | 0.45 | 0.44 | 7.84 | 5.2 |

Enzyme dose = 0.10 % (w/w to dry matter); hydrolysis temperature: ALT 70 °C, GLU/MAL 60 °C. All values are mean values of triplicate measurements. The standard deviations were ≤0.1 for proteins and lipids, ≤0.7 for reducing sugars.

The results showed that dilution of oat slurry influenced the macronutrient composition of oat milk. Protein and lipid contents decreased with the increase in hydromodule. Protein levels dropped from 0.60 % at 1:4 to 0.37 % at 1:7, while lipid content decreased slightly from 0.53 % to 0.45 %.
Reducing sugars concentration also decreased with dilution of the slurry. The highest concentrations of reducing sugars were observed at hydromodule 1:6 (14.2 mg/ml for GLU and 6.6 mg/ml for MAL), indicating optimal enzyme–substrate interaction and a balanced viscosity profile that facilitates substrate accessibility. At a lower hydromodule (1:4), enzymatic activity was limited by restricted diffusivity within the dense matrix, whereas at 1:7, the lower substrate concentration led to low degree of hydrolysis. Therefore, a hydromodule ratio of 1:6, combined with an enzyme concentration of 0.10 % for GLU or MAL, can be identified as the optimal operational condition for enzymatic hydrolysis in oat milk production.

## 4 Conclusion

The present study examined the physicochemical and enzymatic factors influencing oat milk production through enzymatic hydrolysis. Oat flour fraction with fine particles size (75–150 μm) was selected for oat milk production, as it was characterized by high starch and low fiber content, and it provided a homogeneous medium for enzyme hydrolysis. The optimal hydromodule of the oat slurry was determined to be 1:6, based on investigation of the viscosity of the medium and the dry matter yield. At hydromodule 1:6 the best balance between solid loading and enzyme performance was achieved, resulting in largest relative viscosity decrease ($\Delta\eta \approx 90\%$), high dry matter yield (61-65 %) and homogeneous liquefaction of the slurry. Both investigated enzymes GLU and MAL were considered suitable for efficient hydrolysis of oat slurry for oat milk production. Based on the reducing sugars content, the most favorable operational conditions were identified to be 0.10% GLU for 60 min at 70 °C and 0.10% MAL for 60 min at 60 °C. Under these conditions, oat milk, with balanced macronutrient composition, high sugar content, and favorable rheological properties was produced, making it suitable for industrial beverage formulation.

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