Latvia University of Life Sciences and Technologies

Faculty of Agriculture and Food Technology

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Doctoral thesis

ENZYME-ASSISTED OAT PROTEIN CONCENTRATE DEVELOPMENT AND WET EXTRUSION

ENZIMĀTISKĀ AUZU PROTEĪNA KONCENTRĀTA IEGUVE UN MITRĀ EKSTRŪZIJA

for acquiring a Doctoral degree Doctor of science *(Ph.D.)*
in Engineering Sciences and Technologies

NACIONĀLAIS ATTĪSTĪBAS PLĀNS 2020

EIROPAS SAVIENĪBA
Eiropas Sociālais fonds

IEGULDĪJUMS TAVĀ NĀKOTNĒ

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ANNOTATION

The development of the doctoral thesis "Enzyme-assisted oat protein concentrate development and wet extrusion" was carried out from 2020 to 2024 in the laboratories of the Latvia University of Life Sciences and Technologies (LBTU), Faculty of Agriculture and Food Technology, Food Institute, as well as at the Institute of Agricultural Resources and Economics (AREI), LBTU DPP Institute of Horticulture, Latvian State Forest Research Institute "Silava" and JP Biotechnology, Ltd.

The **aim** of the doctoral thesis was to develop enzyme-assisted aqueous extraction methods to obtain oat protein concentrates, evaluate the functional properties of the obtained protein concentrates, and determine their suitability for further processing, including but not limited to wet extrusion.

The **hypothesis:** oat protein concentrate obtained through enzyme-assisted aqueous extraction followed by defatting can be utilised in wet extrusion systems.

Theses confirming the hypothesis:

- oat protein can be extracted and concentrated through enzyme-assisted aqueous extraction;
- the degradation of non-starch polysaccharides during wet enzymatic hydrolysis does not affect the amino acid composition of the oat protein;
- ionic strength influences oat protein aggregation, subsequently affecting its functional properties and the yield of protein recovery;
- defatting methods affect oat protein functional properties;
- defatting the oat protein concentrate improves the wet extrusion process.

Research objects: whole grain oat flakes, fine oat flour, oat protein, oat protein extrudates.

Tasks of the present research are as follows:

- to identify methods suitable for oat protein enzyme-assisted aqueous extraction from commercial whole oat flakes and fine oat flour;
- to identify suitable defatting methods for oat protein concentrates obtained from commercial oats and oat flour;
- to evaluate the redistribution of amino acids in obtained oat protein concentrates and side products, in particular, fibre;
- to evaluate the characteristics of obtained protein concentrates and investigate the functional properties of the obtained oat protein concentrates;
- to determine the extrusion parameters for oat protein concentrate;
- to investigate the physical characteristics of the obtained oat protein extrudate, including its structure, texture, and colour.

Novelty of present research:

- methods for obtaining oat protein concentrate up to 75% (dry matter) through enzyme-assisted aqueous extraction have been developed;
- physiochemical characteristics, amino acid profile and functional properties of oat protein concentrates obtained through enzyme-assisted aqueous extraction have been evaluated;
- technological parameters for the wet extrusion of oat protein concentrate have been determined;
- physical properties of oat protein extrudate obtained through wet extrusion have been studied.

Economic significance of the present research:

- developed methods have shown the possibility of obtaining oat protein concentrates, which are considered as innovative raw materials, potential ingredients for a wide range of food applications, including categories of products with the "clean label";

- functional properties of obtained oat protein concentrates enable producers to determine the applicability of oat protein concentrates in their products;
- defined parameters of the extrusion process and the characteristics of the extrudates derived from oat concentrates support producers in innovating and introducing novel products, specifically extrudates, containing a new ingredient: oat protein concentrate.

The doctoral thesis consists of three chapters.

Chapter 1 describes the oat composition with a focus on protein composition and known methods of protein extraction and concentration. An overview describes the protein-specific properties, their relationship, and performance during extraction and modification, including protein defatting. Attention is paid to functional protein properties and applications. Additionally, suggested protein modifications for improving protein functional properties are reviewed.

Chapter 2 describes the methods and materials used in the thesis.

Chapter 3 presents the results obtained in the study covering oat protein extraction yields, and functional properties of the oat concentrates. The results also cover the field of oat protein extrusion, revealing the parameters enabling extrusion and textural properties of the obtained oat protein extrudate.

The study was partly financed and carried out within the framework of European Social Fund Project No. 8.2.2.0/20/I/001 “LBTU Transition to a new funding model of doctoral studies” and the project “Oat protein in extruded products” funded and implemented by the LBTU (Latvia University of Life Sciences and Technologies) research programme.

The thesis is written in English, and it consists of 115 pages, 12 tables, 30 figures, 8 appendixes, and 249 bibliographic sources.

ANOTĀCIJA

Promocijas darba "Enzimātiskā auzu proteīna koncentrāta ieguve un mitrā ekstrūzija" izstrāde tika veikta no 2020. līdz 2024. gadam Latvijas Biozinātņu un tehnoloģiju universitātes (LBTU) Lauksaimniecības un pārtikas tehnoloģijas fakultātes Pārtikas institūta laboratorijās, kā arī Agroresursu un ekonomikas institūtā (AREI), LBTU APP Dārzkopības institūtā, Latvijas Valsts mežzinātnes institūtā "Silava" un SIA "JP Biotechnology".

*Promocijas darba **mērķis** bija izstrādāt fermentatīvās ūdens ekstrakcijas metodes auzu proteīna koncentrāta iegūšanai, novērtēt iegūto auzu proteīna koncentrātu funkcionālās īpašības un noteikt to piemērotību turpmākai pārstrādei, tostarp, mitrai ekstrūzijai.*

***Hipotēze:** ar fermentatīvās ūdens ekstrakcijas, kam seko attaukošana, palīdzību iegūtu auzu proteīna koncentrātu var izmantot mitrās ekstrūzijas sistēmās.*

***Tēzes**, kas apstiprina hipotēzi:*

- *auzu proteīnu var ekstrahēt un koncentrēt ar fermentatīvās ūdens ekstrakcijas palīdzību;*
- *necietes polisaharīdu noārdīšanās mitrās fermentatīvās hidrolīzes laikā neietekmē auzu proteīna aminoskābju sastāvu;*
- *jonu stiprums ietekmē auzu proteīna agregāciju, kas vēlāk ietekmē tā funkcionālās īpašības un proteīna atgūšanas iznākumu;*
- *attaukošanas metodes ietekmē auzu proteīna funkcionālās īpašības;*
- *auzu proteīna koncentrāta attaukošana uzlabo mitrās ekstrūzijas procesu.*

***Pētījuma objekti:** pilngraudu auzu pārslas, smalkā maluma auzu milti, auzu proteīns, auzu proteīna ekstrudāti.*

*Šim pētījumam ir šādi **uzdevumi**:*

- *identificēt metodes, kas ir piemērotas auzu proteīna fermentatīvai ūdens ekstrakcijai no rūpnieciskām pilngraudu auzu pārslām un smalkā maluma auzu miltiem;*
- *identificēt piemērotas attaukošanas metodes no rūpnieciskām auzām un auzu miltiem iegūtiem auzu proteīna koncentrātiem;*
- *novērtēt aminoskābju pārdalījumu iegūtajos auzu proteīna koncentrātos un blakusproduktos, jo īpaši šķiedrvielās;*
- *novērtēt iegūto proteīna koncentrātu raksturīpašības un izpētīt iegūto auzu proteīna koncentrātu funkcionālās īpašības;*
- *noteikt auzu proteīna koncentrāta ekstrūzijas parametrus;*
- *izpētīt iegūtā auzu proteīna ekstrudāta fizikālās īpašības, tostarp tā struktūru, tekstūru un krāsu.*

*Pētījuma **novitāte**:*

- *ir izstrādātas metodes auzu proteīna koncentrāta ieguvei līdz pat 75% (sausnā) ar fermentatīvās ūdens ekstrakcijas palīdzību;*
- *ir novērtētas ar fermentatīvās ūdens ekstrakcijas palīdzību iegūto auzu proteīna koncentrātu fizikālķīmiskās īpašības, aminoskābju profils un funkcionālās īpašības;*
- *ir noteikti auzu proteīna koncentrāta mitrās ekstrūzijas tehnoloģiskie parametri;*
- *ir izpētītas ar mitrās ekstrūzijas metodi iegūtā auzu proteīna ekstrudāta fizikālās īpašības.*

*Šā pētījuma **ekonomiskā nozīme:***

- *izstrādātās metodes ir pierādījušas iespēju iegūt auzu proteīna koncentrātus, ko uzskata par inovatīvām izejvielām, potenciālām sastāvdaļām plašam pārtikas lietojumu klāstam, tostarp "tīrās etiķetes" produktu kategorijām;*
- *iegūto auzu proteīna koncentrātu funkcionālās īpašības ļauj ražotājiem noteikt auzu proteīna koncentrātu pielietojamību savos produktos;*
- *noteiktie ekstrūzijas procesa parametri un no auzu koncentrātiem iegūto ekstrudātu raksturojums palīdz ražotājiem ieviest jauninājumus un jaunus produktus, jo īpaši ekstrudātus, kas satur jaunu sastāvdaļu–auzu proteīna koncentrātu.*

Promocijas darbā ir trīs nodaļas.

1. nodaļā *ir aprakstīts auzu sastāvs, galveno uzmanību pievēršot proteīna sastāvam un pazīstamajām proteīna ekstrakcijas un koncentrēšanas metodēm. Pārskatā ir aprakstītas proteīnam raksturīgās īpašības, to mijiedarbība un sniegums ekstrakcijas un modificēšanas laikā, tostarp proteīna attaukošanas laikā. Uzmanība pievērsta proteīna funkcionālajām īpašībām un lietojumiem. Papildus tam ir apkopotas atziņas par proteīna modifikāciju proteīna funkcionālo īpašību uzlabošanai.*

2. nodaļā *ir aprakstītas darbā izmantotās metodes un materiāli.*

3. nodaļā *ir sniegti pētījumā iegūtie rezultāti, ietverot auzu proteīna ekstrakcijas iznākumus un auzu koncentrātu funkcionālās īpašības. Rezultāti aptver arī auzu proteīna ekstrūzijas jomu, ir norādīti ekstrūziju sekmējošie parametri un iegūtā auzu proteīna ekstrudāta tekstūras īpašības.*

Pētījums daļēji finansēts un veikts Eiropas Sociālā fonda projekta Nr. 8.2.2.0/20/I/001 "LBTU pāreja uz jauno doktorantūras finansēšanas modeli" un LBTU (Latvijas Biozinātņu un tehnoloģiju universitātes) pētniecības programmas finansētā un īstenotā projekta "Auzu proteīns ekstrudētu produktu ieguvē" ietvaros.

Promocijas darbs ir rakstīts angļu valodā, tajā ir 115 lappuses, 12 tabulas, 30 attēli, 8 pielikumi un 249 bibliogrāfiskie avoti.

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IEGULDĪJUMS TAVĀ NĀKOTNĒ

APPROBATION OF THE RESEARCH / *PĒTĪJUMA APROBĀCIJA*

The research results are summarised and published in 4 peer-reviewed scientific editions in English, which are indexed in the international databases SCOPUS and/or Web of Science. 2 patents have been granted.

Publications:

1. **Sargautis D.**, Kince T., Gramatina I. (2023) Characterisation of the Enzymatically Extracted Oat Protein Concentrate after Defatting and Its Applicability for Wet Extrusion'. *Foods,* 12:2333. https://doi.org/10.3390/foods12122333.
2. **Sargautis D.**, Kince T. (2023) Effect of Enzymatic Pre-Treatment on Oat Flakes Protein Recovery and Properties'. *Foods*, 5:965. https://doi.org/10.3390/foods12050965.
3. Sargautiene V., **Sargautis D.**, Podjava A., Jakobsone I., Nikolajeva V. (2023) Feasibility of Integrating Spray Dried and Freeze Dried Oat β-Glucans in a Synbiotic Formulation with *Akkermansia muciniphila*. *Fermentation*. 9(10): 895. https://doi.org/10.3390/fermentation9100895.
4. **Sargautis D.,** Kince T., Sargautiene V. (2021) Review: Current Trends in Oat Protein Recovery and Utilization in Aqueous Food Systems. *Proceedings of annual 27th International scientific conference Research for Rural Development 2021*. 36:77–83. https://doi.org/10.22616/rrd.27.2021.011.

Granted patents:

1. **Sargautis D.** (2024) A method of producing a drink from an oat material. Latvijas Republikas patentu valde LV 15707, filed on 13 August 2021, and issued 20 February 2024, https://databases.lrpv.gov.lv/patents/LVP2021000048.
2. **Sargautis D.** (2023) A process of producing a plant-based protein. Latvijas Republikas patentu valde LV 15735, filed 5 November 2021, and issued on 20 August 2023, https://databases.lrpv.gov.lv/patents/LVP2021000081.

The research results have been presented at international scientific conferences in Latvia, Lithuania and France.

1. 15th Baltic Conference on Food Science and Technology, FOODBALT-2022 "Food Research and Development in the Baltic States and Beyond", Kaunas, Lithuania. Oral presentation. **Sargautis D.**, Kince T., Gramatina I. Effect of defatting method on enzymatically extracted oat protein solubility (26–27 October 2022).
2. 4th Edition of Euro Global Online Conference on Food Science and Technology, France. Oral presentation. **Sargautis D.,** Kince T., Gramatina I. Evaluation of functional properties of enzymatically extracted oat protein (12–13 September 2022).
3. Workshop within RigaFood 2022 "Innovative and sustainable solutions in food and packaging", Riga, Latvia. Oral presentation. **Sargautis D.**, Kince T., Gramatina I. Oat protein nutritional value reallocation in wet processing (9 September 2022).
4. 27th Annual International Scientific Conference "Research for Rural Development 2021", Jelgava, Latvia. Oral presentation. **Sargautis D.**, Kince T., Sargautiene V. Review: Current Trends in Oat Protein Recovery and Utilization in Aqueous Food Systems (12–13 May 2021).
5. 3rd International Conference "Nutrition and Health", Riga, Latvia. Oral presentation. **Sargautis D**., Kince T., Sargautiene. V. Poster presentation. Sargautiene V., Ligere R., **Sargautis D.** Metabolic activity of the gut microbiota. Investigation of structure formation of oat protein during wet extrusion (9–11 December 2020).

Additional publications released within the duration of the research.

1. Revina O., Avsejenko J., Revins V., **Sargautis D.,** Cirule D., Valdovska A. (2020) Effect of dietary supplementation with β-glucan on growth performance and skin-mucus microbiota of sea trout (*Salmo trutta*). *Fisheries & Aquatic Life*. 28:155–165. https://doi.org/10.2478/aopf-2020-0019.

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LIST OF DESIGNATIONS AND ABBREVIATIONS INCLUDED IN PHD THESIS / *PROMOCIJAS DARBĀ IEKĻAUTO APZĪMĒJUMU UN SAĪSINĀJUMU SARAKSTS*

A1	Oat protein dried, obtained by treating oat flakes with α-amylase / *Kaltēts auzu proteīns, kas iegūts, apstrādājot auzu pārslas ar α-amilāzi*
AF1	Oat fibre, obtained in the process of treating oat flakes with α-amylase / *Auzu šķiedrvielas, kas iegūtas, apstrādājot auzu pārslas ar α-amilāzi*
Ala	Alanine / *Alanīns*
ANOVA	Analysis of variance / *Dispersijas analīze*
AR1	Oat protein, obtained by treating oat flakes with alpha-amylase changing ionic strength / *Auzu proteīns, kas iegūts, apstrādājot auzu pārslas ar α-amilāzi, mainot jonu stiprumu*
AREI	DPP "Institute of Agricultural Resources and Economics" / *Agroresursu un ekonomikas institūts*
Arg	Arginine / *Arginīns*
Asp	Aspartic acid / *Asparagīnskābe*
AX1	Oat protein dried, obtained by treating oat flakes with alpha-amylase and complex enzyme for non-starch polysaccharides / *Kaltēts auzu proteīns, kas iegūts, apstrādājot auzu pārslas ar α-amilāzi un komplekso enzīmu cieti nesaturošiem polisaharīdiem*
AXF1	Oat fibre, obtained in the process of treating oat flakes with alpha-amylase and complex enzymes for non-starch polysaccharides / *Auzu šķiedrvielas, kas iegūtas, apstrādājot auzu pārslas ar α-amilāzi un kompleksiem enzīmiem cieti nesaturošiem polisaharīdiem*
AXR1	Oat fibre, obtained in the process of treating oat flakes with alpha-amylase and complex enzymes for non-starch polysaccharides / *Auzu šķiedrvielas, kas iegūtas, apstrādājot auzu pārslas ar alfa amilāzi un kompleksiem enzīmiem cieti nesaturošiem polisaharīdiem*
Conc.	Concentrate / *Koncentrāts*
Cys	Cysteine / *Cisteīns*
C-1	Carbon number one / *Ogleklis numur viens*
DES	Deep eutectic solvent / *Dziļais eitektiskais šķīdinātājs*
FAO	Food and Agriculture Organization of the United Nations / *Apvienoto Nāciju Pārtikas un lauksaimniecības organizācija*
FL1	Whole oat flakes / *Pilngraudu auzu pārslas*
Glu	Glutamic acid / *Glutamīnskābe*
Gly	Glycine / *Glicīns*
HCl	Hydrochloric acid / *Sālsskābe*
His	Histidine / *Histidīns*
Ile	Isoleucine / *Izoleicīns*
LBTU	Latvia University of Life Sciences and Technologies / *Latvijas Biozinātņu un tehnoloģiju universitāte*
Leu	Leucine / *Leicīns*
Lys	Lysine / *Lizīns*
Met	Methionine / *Metionīns*
Mr	Relative molecular mass / *Relatīvā molekulmasa*
MW	Molecular weight / *Molekulmasa*
NSI	Nitrogen solubility index / *Slāpekļa šķīdības indekss*
NSP	Non-starch polysaccharides / *Cieti nesaturošie poliosaharīdi*

OC1	Oat protein concentrate obtained from fine flour before defatting / *Auzu proteīna koncentrāts, kas iegūts no smalkiem miltiem pirms attaukošanas*
ODC1	Oat protein concentrate OC1 defatted by supercritical CO_2 / *Auzu proteīna koncentrāts OC1, kas attaukots ar superkritisko CO_2*
ODE1	Oat protein concentrate OC1 defatted by ethanol / *Auzu proteīna koncentrāts OC1, kas attaukots ar etanolu*
OF1	Oat flour with reduced fibre content / *Auzu milti ar samazinātu šķiedrvielu saturu*
Phe	Phenylalanine / *Fenilalanīns*
p*I*	Isoelectric point / *Izoelektriskais punkts*
pK	Acid dissociation constant / *Skābes disociācijas konstante*
Pro	Proline / *Prolīns*
rpm	Revolutions per minute / *Apgriezieni minūtē*
SC-CO_2	Supercritical CO_2 / S*uperkritiskais CO_2*
SDS-Page	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis / *Nātrija dodecilsulfāta-poliakrilamīda gēla elektroforēze*
Ser	Serine / *Serīns*
SILAVA	Latvian State Forest Research Institute “Silava” / *Latvijas Valsts mežzinātnes institūts “Silava”*
Thr	Threonine / *Treonīns*
Tris HCl	Tris(hydroxymethyl)aminomethane / *Tris(hidroksimetil)aminometāns*
Tyr	Tyrosine / *Tirozīns*
Val	Valine / *Valīns*
wt.	Weight / *Svars*

INTRODUCTION / *IEVADS*

The increasing popularity of plant-based products and the anticipated growth in plant-based protein consumption have generated a demand for high-quality ingredients in various applications, such as replacements for meat (Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017), milk (Craig & Fresán, 2021), eggs, and fish (McClements & Grossmann, 2021). While several plant proteins such as wheat, soy, and pea are readily available and favoured for their cost-effectiveness and functional properties, oat protein has not been fully utilised. Oats, recognised for their exceptional nutritional value and amino acid composition (Abdellatif Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009), show promise as an alternative source for plant protein concentrates and isolates. Oats contain higher protein levels in their groats compared to other cereals (Klose & Arendt, 2012) and are well-suited for cultivation in the local region where this research was conducted (Sterna, Zute, & Brunava, 2016). Nevertheless, despite the appeal of oat protein, its commercial production in concentrate and isolate forms remains limited.

Despite the extensive study of oat protein characteristics (Spaen & Silva, 2021), the current methods for extracting oat protein frequently employ harsh alkaline extraction followed by precipitation, potentially altering the protein's properties, and affecting its technological functionality and nutritional value. The precipitation method may also pose challenges in industrial applications, particularly in dealing with by-products during protein recovery. An alternative method to concentrate protein relies on air separation, which is inherent to the milling process (Sibakov, 2014). This approach could also be considered a by-product of the oat β-glucan concentration process. However, dry concentration methods do not guarantee protein concentrates free from suspended solids, thereby limiting their applicability to specific areas.

Another way to concentrate oat protein is through enzyme-assisted aqueous extraction. This method can produce a versatile protein concentrate that can be used in various applications. It allows for the adjustment of protein concentration levels and control of suspended solids. Additionally, the resulting oat protein concentrate can be used as a valuable raw material for developing unique products, such as oat protein extrudates.

The **aim** of the doctoral thesis was to develop enzyme-assisted aqueous extraction methods to obtain oat protein concentrates, evaluate the functional properties of the obtained protein concentrates, and determine their suitability for further processing, including but not limited to wet extrusion.

The **hypothesis:** oat protein concentrate obtained through enzyme-assisted aqueous extraction followed by defatting can be utilised in wet extrusion systems.

Theses confirming hypothesis:

- oat protein can be extracted and concentrated through enzyme-assisted aqueous extraction;
- the degradation of non-starch polysaccharides during wet enzymatic hydrolysis does not affect the amino acid composition of the oat protein;
- ionic strength influences oat protein aggregation, subsequently affecting its functional properties and the yield of protein recovery;
- defatting methods affect oat protein functional properties;
- defatting the oat protein concentrate improves the wet extrusion process.

Research objects: whole grain oat flakes, fine oat flour, oat protein, oat protein extrudates.

Tasks of the present research are as follows:

- to identify methods suitable for oat protein enzyme-assisted aqueous extraction from commercial whole oat flakes and fine oat flour;
- to identify suitable defatting methods for oat protein concentrates obtained from commercial oats and oat flour;

- to evaluate the redistribution of amino acids in obtained oat protein concentrates and side products, in particular, fibre;
- to evaluate the characteristics of obtained protein concentrates and investigate the functional properties of obtained oat protein concentrates;
- to determine the extrusion parameters for oat protein concentrate;
- to investigate the physical characteristics of the obtained oat protein extrudate, including its structure, texture and colour.

Novelty of present research:

- methods for obtaining oat protein concentrate up to 75% (dry matter) through enzyme-assisted aqueous extraction have been developed;
- physiochemical characteristics, amino acid profile and functional properties of oat protein concentrates obtained through enzyme-assisted aqueous extraction have been evaluated;
- technological parameters for the wet extrusion of oat protein concentrate have been determined;
- physical properties of oat protein extrudate obtained through wet extrusion have been studied.

Economic significance of the present research:

- developed methods have shown the possibility to obtain oat protein concentrates, which are considered as innovative raw materials, potential ingredients for a wide range of food applications, including categories of products with the “clean label”;
- functional properties of obtained oat protein concentrates enable producers to determine the applicability of oat protein concentrates in their products;
- defined parameters of the extrusion process and the characteristics of the extrudates derived from oat concentrates support producers in innovating and introducing novel products, specifically extrudates, containing a new ingredient: oat protein concentrate.

1. LITERATURE REVIEW / *LITERATŪRAS APSKATS*

1.1 Plant-based protein and its potentiality / *Augu izcelsmes proteīns un tā potenciāls*

1.1.1. General characteristics of oat grains / *Auzu graudu vispārīgs raksturojums*

Oats (*Avena sativa*) represent a significant raw material in the context of human food production. Global oat production reached 25 million tonnes in 2023 (European Commission, 2023). Among the approximately 70 oat species identified, *Avena sativa* (hulled) and *Avena nuda* (naked oats) are the most cultivated. Oats possess various favourable qualities, including their ability to thrive with reduced fertiliser requirements compared to crops such as wheat or corn, as well as their adaptability to colder climates (Rasane, Jha, Sabikhi, Kumar, & Unnikrishnan, 2015). The forthcoming research will predominantly focus on hulled oats.

In Latvia, husked breeding lines of oats typically exhibit an average composition of 10.58% protein, 5.15% fat, 48.10% starch, and 17.60% total dietary fibre (Sterna et al., 2016). A comprehensive study that covered data from 975 oat germplasm samples collected from diverse global regions reported average nutritional components, including a crude fibre content of 2.14%, lipid content of 4.73%, and β-glucan content of 3.05% (Rauf et al., 2019). Furthermore, an investigation based on compiled information from the U.S. Department of Agriculture's Agricultural Research Service provided proximate values for oat constituents. These mean values were as follows: water content at 8.5%, carbohydrates at 58.7%, protein at 14.0%, fat at 8.0%, dietary fibre at 9.0%, and ash at 1.8% (Welch, 2011).

The forthcoming section provides an in-depth review of oat protein and its formation and localisation. This is followed by sections that outline the main oat constituents such as starch, fibre, and fat–the components linked to the protein extraction process, a subject that will be elaborated on in subsequent discussions.

1.1.2. Oat protein / *Auzu proteīns*

Protein is essential for the growth and maintenance of living organisms, playing a pivotal role in sustaining the body's nitrogenous compounds. Providing an adequate and efficiently utilised amount of protein is a fundamental goal in food supply systems. Plant-based protein sources are particularly significant, constituting at least 60% of the protein consumed in the diet (Kawakatsu & Takaiwa, 2017; Krishnan & Coe, 2001). Plant seeds typically accumulate protein in the range of 7% for monocotyledonous plants and up to 40% for dicotyledonous plants (Shewry, Napier, & Tatham, 1995; Krishnan & Coe, 2001). Cereal proteins, as a category, are the most important, accounting for approximately 40% of the world's protein consumption (Kawakatsu & Takaiwa, 2017).

Proteins in seeds are primarily stored as a reservoir of amino acids for seed germination and growth. Within this protein storage, certain groups dominate, which define protein structure, functionality, nutritional value, and end-use applications. In monocotyledonous plants, protein bodies are primarily deposited in the endosperm and accumulate alcohol-soluble proteins, except for in the case of oats and rice, where the primary protein is salt-soluble. Typically, classification using the Osborne fractionation method categorises major cereal seed proteins into alcohol-soluble prolamins (Walburg & Larkins, 1983). Oat seeds predominantly store protein as globulin, similar to legumes, with an amino acid profile that is more nutritionally valuable when compared to glutelin-rich crops like wheat or corn. Moreover, their health benefits and suitability for cultivation make oats particularly attractive. The amino acid composition of oat globulin is akin to soy glycinin, with some variations such as higher levels of tyrosine and phenylalanine and lower levels of asparagine/aspartic acid, proline, and lysine (Brinegar & Peterson, 1982).

The availability of sulphur is essential for amino acids like cysteine and methionine, which are required for protein synthesis (Shewry et al., 1995). The main storage protein in oats

is 11S globulin-like, while prolamins account for about 5 to 10% of the total protein in seeds. Protein bodies are discrete deposits where proteins are stored, and Shewry (1995) noted that protein fractions exhibit polymorphism.

Understanding storage proteins is crucial for harnessing novel protein structures for consumer products or agricultural applications. It enables the efficient utilisation of proteins without interfering with the biological processes. Oats require minimal cultivation input and positively influence soil structure when incorporated into crop rotation (Smulders et al., 2018). The protein content in oats can vary significantly depending on the variety and growing region. For example, Kourimska et al. (2018) reported protein content in oats cultivated in the Czech Republic, which varied from 13.9% to 18.4%. Similarly, Sterna et al. (2016) found that protein content in Latvian oat varieties ranged from 10.6% to 15.7% for husked and naked oats, respectively.

In the paragraphs below, oat protein will be characterised in greater detail.

Prolamins (*Avenins*)

Prolamins are typically characterised by their high glutamine and proline content and are primarily classified based on their solubility in aqueous alcohol mixtures. However, the classification according to the Osborne method does not yield definitive amino acid data. Shewry and colleagues (1995) proposed that the definition of prolamins should be expanded, as not all prolamins exhibit solubility in alcohol-water mixtures. Amino acid sequence comparisons also do not yield absolute data, as some portions of prolamins are linked by inter-chain disulphide bonds, rendering them insoluble in aqueous alcohol solutions. In their study, Nałęcz et al. (2017) presented a modified method that involved the extraction of total proteins and sequential Osborne fractionation, which was based on solubility differences. This method facilitated the isolation and subsequent characterisation of prolamins using the two-dimensional polyacrylamide gel electrophoresis (2-DE) technique. Initially, proteins were separated based on their isoelectric point (p*I*), followed by electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS) gels (Nalecz, Szerszunowicz, Dziuba, & Minkiewicz, 2017). When applied to the Flämingstern variety, Nałęcz and colleagues (2017) identified oat prolamins with a molecular weight (MW) range of 19.9 to 25.7 kDa and isoelectric points (p*I*) ranging from 6.0 to 8.4. In contrast, prolamins from *Triticeae* species were found to exhibit greater polymorphism, with molecular weights (Mr) ranging from approximately 30 000 to 90 000 (Shewry et al., 1995). One of the avenins was isolated and characterised by Egorov (1988). Designated as avenin N9 it was isolated from oat variety Narymsky 943 and consisted of 182 amino acid residues with calculated Mr of 21 000. It has a unique N-terminal amino acid sequence (residues 1–10) and contains three conserved regions: A (residues 43–67), B (residues 86–121) and C (residues 150–174), which are present in all the S-rich and high molecular weight prolamins. It differed from S-rich α-gliadins of wheat by a lack of polyglutamine regions.

In general, avenins exhibit an average and intermediate protein composition compared to other cereals. Oat avenins, similar to rice or corn prolamins, are rich in leucine (11 mol%) and valine (8 mol%), although proline (10 mol%) was detected at a relatively low extent (Radomír Lásztity, 1996). Furthermore, the amino acid profile of oat prolamins is characterised by a low content of basic amino acids and a high content of glutamic acid (including glutamine, which can reach up to 36.1% (Radomir Lásztity, 1998)).

Albumins

The majority of metabolically active proteins in oats are represented by albumins, which account for approximately 1−12% of the total protein content. Runyon et al. (2015) reported the presence of albumins at a level of 17.60% of the total protein. However, even higher content, reaching a level of 23.37% of the total protein, was recently reported in oat grains of the Drug

variety (Kriger, Kashirskikh, Babich, & Noskova, 2018). It was found that albumins mainly consist of monomeric proteins, and they represent a water-soluble fraction (Klose, Schehl, & Arendt, 2009). Typically, albumins have been classified as a group of proteins with a sedimentation coefficient of approximately two (Youle & Huang, 1981).

Oat albumins are distinguished by their notably high lysine content compared to other cereal albumins (Ercili-Cura et al., 2015). It has been observed that albumins denature at lower temperatures than globulins, typically within the range of 87–95 °C (Kaukonen et al., 2011; Pori, Nisov, & Nordlund, 2022). The reported molecular weight of oat albumins varies within the range of 14–17 kDa, 20–27 kDa, and 36–47 kDa (Klose et al., 2009). Bands of proteins in the 36–47 kDa range have been observed to decrease during the malting process. Additionally, peaks in the albumin fraction at 6–9 kDa were reported using LabChip, which were undetectable with commonly employed gel electrophoresis techniques (Klose et al., 2009).

The isoelectric points of albumins range from pH 5 to 8. The majority of metabolically active proteins are typically found in the albumin fraction, and these proteins are generally classified as enzymes. Oats have been found to contain numerous enzyme activities, including α-amylase, phosphatase, tyrosinase, maltase, protease, lichenase, phenoxyacetic acid hydroxylase and lipase (Radomír Lásztity, 1996). However, it should be noted that lipase activity is typically inactivated by thermal treatment, as it is considered to initiate the degradation of lipids in oats. This is essential, as lipid hydrolysis and oxidation could lead to rancidity (Keying, Changzhong, & Zaigui, 2009). Oat albumins are characterised by their high concentrations of lysine, asparagine-aspartic acid, and alanine, averaging 8.3, 12.5, and 7.1%, respectively. In contrast, the albumin fraction has the lowest content of glutamine-glutamic acid, accounting for only 15.1% of the total amino acids in albumins (Radomír Lásztity, 1996).

Globulins

Oat globulins, which are closely related to dicot 11S globulins, are believed to accumulate in a similar manner. Burges and colleagues (1983) extracted and identified three primary fractions of oat globulin. Dissolved in 3.0 mL 0.05 *M* Tris/HCl pH 8.0, 1.0 *M* NaCl globulin preparations were layered on top of 5–20% w/w linear sucrose gradient made up in the same buffer. Through centrifugation, the globulins were separated into fractions with sedimentation coefficients of approximately 3S, 7S, and 12S. Notably, the majority of the separated globulins belonged to the 12S sedimentation coefficient fraction (Burgess et al., 1983).

Support for this assertion is derived from Peterson's work (1978), which demonstrated that the predominant oat protein fraction, globulin, possessed a sedimentation coefficient of 12.1. Furthermore, the molecular weight of the globulin was determined to fall within the range of 320 000. It was postulated that globulin consisted of two subunits, with molecular weights of 21 700 and 31 700, and a model was proposed suggesting the presence of six of each of these subunits per polypeptide of the globulin. Subsequently, Brinegar and Peterson (1982), as well as Walburg and Larkins (1983) conducted further characterisations of oat globulins employing various electrophoretic techniques. Their work provided precise molecular weight specifications for the α and β polypeptides (nomenclature was based on polypeptides' weight and pI), which were found to range from 32 500 to 37 500 and from 22 000 to 24 000, respectively (Brinegar & Peterson, 1982). Walker and Larkins (1983) additionally confirmed the presence of two distinct classes of oat globulin, with molecular weight ranges of 20 000 to 25 000 and 35 000 to 40 000. A similar observation was reported by Yue et al. (2021), who confirmed two predominant bands at ~36 and 22 kDa. These bands correspond to acid and the basic subunit of oat 12S globulin. The molecular weight of the holoprotein was determined to be within the range of 327 000 to 369 000 (Brinegar & Peterson, 1982). Furthermore, the determined pI_{25}^{α} for α was 5.9–7.2 while β polypeptides fell in the pI_{25}^{α} range of 8.7–9.2.

Comparing the amino acid profiles of both groups separately with the initial oat globulin and soy glycinin revealed differences between the α and β groups. Specifically, the α group

exhibited significantly higher levels of glutamine/glutamic acid, glycine, and tryptophan, while basic amino acids, asparagine/aspartic acid, and methionine predominated in the β group. The amino acid composition of oat globulin showed similarities to soy glycinin, with the exceptions of tyrosine and phenylalanine, which were found in higher amounts in oat globulin, and asparagine/aspartic acid, proline, and lysine, which were present in lower amounts. In addition, it was suggested that disulphide links exist between the α and β polypeptides. This hypothesis was based on the observation that when the globulin was not reduced before electrophoresis, the α and β polypeptides were not detected. However, the existence of polypeptide formations in the molecular weight range of Mr 53 000–58 000 was evident (Brinegar & Peterson, 1982).

Comparing globulins to other oat fractions, it is noteworthy that globulins are characterised by a high arginine content, falling within the range of 8.5% to 9.2% of the globulin fraction (Klose et al., 2009; Radomir Lásztity, 1998).

Glutelin

Nnanna and Gupta (1996) conducted an investigation into the characterisation of oat bran protein, specifically focusing on oat bran protein globulins. In contrast to what has been mentioned previously, their findings indicated that glutelin was the predominant component in the oat bran protein fraction. Despite the application of an improved isolation method, which was based on previously well-characterised isolation methods (Peterson, 1978; Yung Ma, 1983), the research results revealed that the content of glutelin, globulin, prolamin, and albumin was 62.5%, 23.0%, 1.1%, and 2.1%, respectively. These results were both unexpected and challenging to interpret. The authors analysed the secondary structure of oat bran globulin, revealing a predominance of α-helices (49.6%), followed by β-sheets (42.9%), with a negligible presence of a random coiled secondary structure. This structural composition was notably distinct from soy 7S, which predominantly exhibited a random coiled secondary structure (71.9%).

The elevated glutelin content was similarly reported by Kriger et al. (2018). In their study, glutelin content was determined to be 27.98% in the oat variety Drug and 33.76% in Adamo. It was also noted that even higher levels of glutelin were present in the Rysakt variety, although specific values were not provided. The globulin content represented the second-largest fraction of proteins. Additionally, an unexpectedly high content of albumins was observed, exceeding 23% of the total protein content in the Drug variety.

Typically, glutelin is quantified as a protein fraction obtained through extraction with either acidic or basic solutions after the removal of albumins (the water-soluble fraction), globulins (the salt-soluble fraction), and prolamins (the alcohol-soluble fraction). It has been noted that these extraction methods are not entirely comprehensive, as some nitrogen remains in the samples. Following the extraction of the aforementioned fractions, glutelin can be completely solubilised using alkaline solutions containing sodium dodecyl sulphate and 2-mercaptoethanol. However, reported results have shown inconsistencies, ranging from 5% to 66% glutelin content (Radomír Lásztity, 1996).

Utilising two-dimensional electrophoresis, which includes isoelectric focusing (IEF) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), it has been revealed that the majority of polypeptides present in the residual fraction exhibit co-migration patterns consistent with prolamins and, notably, globulins. Nevertheless, a subset of minor polypeptide groups observed did not demonstrate corresponding electrophoretic profiles with either prolamins or globulins. It is plausible that these minor proteins represent oat glutelin. Correspondingly, the amino acid profile of oat glutelin falls within the intermediate range in comparison to other fractions, with no distinctive predominance of specific amino acids (Radomír Lásztity, 1996).

Oat protein formation and localisation

Early reports by Lending and colleagues (1989) concluded that the most oat globulin aggregates could be found within the vacuolar protein bodies, whereas the most avenins aggregate within the rough endoplasmic reticulum. It appears that avenins initially form protein aggregates inside the rough endoplasmic reticulum, which may subsequently be transported to the vacuole. Shewry and colleagues (1995) speculated that the observed phenomenon occurs due to the interaction of individual prolamin molecules, which subsequently form insoluble conglomerations retained in the endoplasmic reticulum (ER) lumen.

Lending et al. (1989) also reported that they could not confirm the involvement of the Golgi apparatus in protein formation in oat endosperm. This speculation was supported by earlier work by Saigo et al. (1983), who confirmed the infrequent or unobserved appearance of dictyosomes in the electron micrographs of the oat material they studied. Additionally, direct connections between the rough endoplasmic reticulum and the smooth vacuolar membrane were observed in developing oats. This led to the assumption that the formation of protein bodies might be possible through direct transport between the rough endoplasmic reticulum and the vacuole. However, it is apparent that despite the spatial occurrence and accumulation size of proteins, there is an intermixing of protein fractions in oats.

The transformation of oat proteins during malting was investigated by Klose et al. (2009). A comparison of free amino acid content in un-malted and malted oats did not reveal significant differences. However, it was observed that tyrosine decreased by 57%, while histidine increased by 20%. When the proteins were fractionated using the Osborne method and subsequently analysed using the Lab-on-a-Chip technique, which allows for the separation of proteins by their molecular weight, it was found that albumins increased in all amino acids except for arginine and histidine. The 12S globulins were only slightly affected, while the amount of 7S globulins decreased. Globulins exhibited an increase in lysine content, yet the lysine content decreased significantly by 28%. Prolamins were completely degraded, while glutelin, identified as the remaining fraction with a 9 kDa polypeptide, remained unchanged. In summary, the study revealed that malting induces the degradation of oat proteins into small peptides and amino acids (Klose et al., 2009).

1.1.3. Oat constituents beyond protein / *Auzu sastāvdaļas ārpus proteīna*

Starch

Starch constitutes the primary component of oats, with a starch content that can reach up to 60% (Zhu, 2017). Oat starch comprises two major polysaccharides, amylose (averaged MW 1.68×10^5) and amylopectin with a weigh-averaged molecular weight of 1.36×10^7 and 3.19×10^6 for larger and smaller fractions, respectively. Wang and White (1994) reported a third intermediate material in oats, characterised by higher iodine affinity values than amylopectin. Despite having a lower molecular weight than amylose, this material exhibited a structure closer to amylopectin.

The proportion of amylose, a linear molecule, in starches, can vary and typically ranges from 17.3% to 33.6% (Sowa & White, 1992; Whistler & BeMiller, 2009). Amylopectin, on the other hand, exhibits branching at one point for every 20–25 straight residues (Sayar & White, 2011). Debranched starches have been reported to possess degrees of polymerisation with chain lengths of 593–703 for amylose, 42–44 for amylopectin (long-chain amylopectin), and 17–22 for short-chain amylopectin. Starches in oats may contain a relatively high lipid content, typically ranging from 0.67% to 2.50% in native starches (Sowa & White, 1992; Whistler & BeMiller, 2009). The temperature of gelatinisation at its peak has been reported to be approximately 62.5 °C (Whistler & BeMiller, 2009). Additionally, it has been observed that oat starch retrogrades to a lesser extent compared to high amylose starches such as corn, which may be attributed to its higher lipid content (Whistler & BeMiller, 2009). Some other

noteworthy unique properties of oat starch could be addressed to the relatively small granule size of 3–10 μm, and high crystallinity (Punia et al., 2020; Sowa & White, 1992).

The strong protein starch matrix makes oat starch difficult to separate (Sayar & White, 2011). The starch isolation process assumes protein removal through alkaline solubilisation in the presence of proteolytic and cellulolytic enzymes. However, due to developed bran hydration and the presence of protein layers, its industrial implementation has had limited success (Autio & Eliasson, 2009).

Fibre

Fibre is primarily concentrated in the oat husks, bran, and aleurone layer. Oat husks were found to contain approximately 91.1% fibre, while oat bran accounted for 23.6% by dry weight (Dziki, Gawlik-Dziki, Tarasiuk, & Różyło, 2022). Generally, fibre is considered as a constituent comprising carbohydrates, excluding digestible components, in particular starch. Due to the complexity of carbohydrates, various methods are employed to determine the fibre content. The major constituents of fibre are indigestible polysaccharide components, typically including cellulose, hemicellulose, and gums (Welch, 2011). Cellulose and some hemicelluloses are water-insoluble, while gums and certain hemicelluloses are water-soluble. Insoluble dietary fibre consists of resistant starch, non-starch polysaccharides, Klason lignin, which comprises lignin, modified lignin, unavailable cell wall protein, polymers formed during the Maillard reaction, and tannin-protein complexes (AACC Report, 2001; Manthey, Hareland, & Huseby, 1999).

An extensive report by Englyst et al. (1989) indicated that oatmeal contains, on average, 7.7% non-starch polysaccharides, of which the soluble portion accounts for 58% of the total determined non-starch polysaccharides. The insoluble non-starch polysaccharides constitute 3.2%, with cellulose averaging 0.6%. The major monosaccharides identified in total oatmeal non-starch polysaccharides are glucose (65%), xylose (17%), arabinose (12%), galactose (2%), mannose (1%), and uronic acids (3%), respectively. Soluble fibre is predominantly composed of glucose (87%), with the other mentioned monosaccharides each accounting for less than 4%. The distribution of monosaccharides in insoluble fibre exhibits a distinct pattern, with glucose (35%), xylose (33%), and arabinose (23%) being the primary constituents, while galactose, mannose, and uronic acids are present in trace amounts in oatmeal.

While the predominant insoluble polysaccharide, cellulose, consists solely of (1→4) - β-D linkages and exhibits a highly crystalline and insoluble structure, β-glucan, a non-starch polysaccharide consisting of β-d-glucopyranosyl units, which are joined by β - (1→4) and β - (1→3) linkages, exists in both soluble and insoluble forms. It is believed that the water-unextractable β-glucan along with arabinoxylan form covalent structures between themselves and each other, as arabinoxylans are reported to be linked with phenolic compounds in the cell wall
(W. Cui & Wood, 2000). Additionally, these polysaccharides have the capacity to interact with other materials such as lignin and cellulose (Virkki, Johansson, Ylinen, Maunu, & Ekholm, 2005). Moreover, the solubility of oat β-glucan is highly dependent on the extraction techniques employed (Welch, 2011). Reported proximate values for β-glucan typically range from 2.0 to 7.0% (W. Cui & Wood, 2000), with the most common values falling within the range of 4.5% to 5.5% (Wood, 2011). These values closely align with those reported by Sterna et al. (Sterna et al., 2016) which averaged 3.15% for husked oats and 3.29% for naked oats. The reported molecular weight (MW) for oat β-glucan is one of the highest, reaching up to 4×10^6 (W. Cui & Wood, 2000). Due to its high MW, this polysaccharide falls into the category of viscoelastic fluids. It has been speculated that the presence of charged groups, even at low levels, might affect its rheological characteristics (W. Cui & Wood, 2000).

Lipids

Oat lipids mainly consist of unsaturated fatty acids (Zhu, 2017), with a total lipid content that can reach up to 15.5% (Whistler & BeMiller, 2009). Specific breeding could increase lipid content up to 18.1%, although an observable decrease was reported in agronomic properties (Lehtinen & Kaukovirta-Norja, 2011). Typically, palmitic (16:0), oleic (18:1) and linoleic (18:2) acids count for 90–95% of the total fatty acids in oat, and their composition is also influenced by the growth conditions. Lipid class composition reveals that 50–85% of total lipids are acylglycerols, with triacylglycerols being the predominant form, and 20–40% polar phospho- and glycolipids (Lehtinen & Kaukovirta-Norja, 2011).

Internal starch lipids are observable within the starch granules, either surrounded by the amylose helix or in spaces between the amylose and amylopectin (Sayar & White, 2011). These lipids are referred to as "true" starch lipids. Lipids are also found on the surface of the starch granules, mainly consisting of monoacyl lipids, and "non-starch" lipids originating from the endosperm (Sayar & White, 2011). The starchy endosperm contains the majority of oat lipids. In the starchy endosperm, oil bodies are typically not discerned as separate entities but appear to meld into each other and become embedded within the matrix formed by the starch and protein compounds. In contrast, discrete lipid bodies can be observed in the aleurone layer, scutellum and embryo (Heneen et al., 2008). The reported redistribution of oat lipids in the endosperm averages 53.3% of the total lipid content in the groat, although the concentration of lipids in the endosperm is relatively low, accounting for about 6.0% and 1.0% of free (extracted by nonpolar solvents) and bound lipids (extracted by polar solvents), respectively (M. Zhou, Robards, Glennie-Holmes, & Helliwell, 1999). The lipid concentration in the scutellum and embryonic axis has been reported to be significantly higher, averaging 24.0% for the scutellum and 15.3% for the embryonic axis. However, the redistribution of total lipids in the aforementioned fractions of the groat was relatively low, accounting for 6.4% of the embryonic axis and 2.1% of the scutellum, respectively (M. Zhou et al., 1999).

The industrial wet oat fractioning process, which involves oat milling and soaking in water, can disrupt cellular structures, leading to the rapid development of unpleasant flavours that can spoil the quality of bran, protein, or starch fractions. This deterioration of flavour is often caused by the degradation of esterified fatty acids. Wet fractionation of oats into fibre, starch, and protein fractions has been shown to alter the lipid classes (Liukkonen, Montfoort, & Laakso, 1992). An increase in free fatty acids has been observed, primarily due to the hydrolysis of triglycerides, likely resulting from inadequate enzyme deactivation. Additionally, mechanical damage during oat processing has been reported as a factor that increases the levels of free fatty acids in oats. Intensive heating may be necessary to prevent triglyceride hydrolysis (Liukkonen et al., 1992).

Minor oat compounds

Phytic acid in oats typically accounts for about 0.5–1.3% by weight and its concentration is influenced by factors such as fertilisation and environment (H. Li, Qiu, Liu, Ren, & Li, 2014; G. Miller, Youngs, & Oplinger, 1980; Rivera-Reyes et al., 2009; Saastamoinen, Plaami, & Kumpulainen, 1992). It is primarily concentrated in the aleurone layer of oats, which is typically transferred to the bran stream during oat dry fractionation. The amount of phytic acid in oat brans typically ranges from 27% to 53% of total phytic acid content, while its redistribution is affected by the milling process (H. Li et al., 2014). The phytic acid amount was found to be positively correlated with the protein content in oats (r=0.959, (Saastamoinen et al., 1992)).

Minerals such as phosphorus, potassium, magnesium and calcium dominate in oats, counting as representative values in fresh grain at 389, 459, 145, and 54 mg 100 g^{-1}, respectively (Welch, 2011). The availability of minerals in oats can be substantially influenced by their presence in the soil. Oats are high in vitamin E, wherein α-tocopherol and α-tocotrienol

comprise the major forms of it (Rasane et al., 2015). Oats are famous for vitamins such as thiamine, niacin and riboflavin (Zhu, 2017), with these elements found in higher amounts compared to other cereals (Welch, 2011).

Phenolic compounds including phenolic acids such as ferulic, p-coumaric, caffeic, vanillic, and hydroxybenzoic acid have been detected in oats. Additionally, trace elements such as flavonoids including glycosyl-vitexin, apigenin and others, have been identified in oats. A comprehensive review of oat phenolics was provided by Collins (2011). Furthermore, oats contain unique components not found in other grains, such as phenolic alkaloids in the form of avenanthramides (Zhu, 2017).

1.2. Plant-based protein concentration methods / *Augu izcelsmes proteīnu koncentrēšanas metodes*

The extraction of plant-based protein is generally considered as an initial step followed by protein purification. However, the release of proteins into a medium suitable for further purification or breaking grain kernel cells in preparation for further downstream applications assumes protein separation from the major kernel compounds, which typically reveals varying degrees of interaction with one another.

The objective of this chapter is to review the possible interactions between the major oat kernel components and techniques typically involved in plant-protein extraction and purification. Much of the literature is based on alternative plant proteins, related to oat protein to some extent, as little attention has been given to research on oat protein extraction methods distinct from the conventional alkaline extraction approach.

1.2.1. Interactions between oat kernel components / *Mijiedarbība starp auzu grauda komponentiem*

Starch–lipids–protein complexes

The understanding of interactions within starch-protein-lipid complexes is currently limited and emerging (S. Wang et al., 2020). However, these interactions hold significant importance, as they are essential for purifying or modifying these compounds to achieve specific physicochemical characteristics. Interpreting these interactions can be facilitated by considering the behaviours of substances with similar or closely related properties. Figure 1.1. illustrates the distribution of oat components within the oat grain structure.

Heneen et al. (2008) investigated the fusion of oil bodies in oats. The urea-washed oil fractions obtained from oats were found to contain oil-body-associated proteins. These associated proteins, separated by SDS-PAGE, primarily consisted of proteins with molecular weights of 14, 16, and 28 kDa. Notably, the 16 kDa protein was predominant in the embryo and scutellum, while the 28 kDa protein was primarily located in the endosperm. The 14 and 16 kDa proteins were identified as specific lipid-binding proteins known as oleosins. The reported amount of bound lipids to protein in oat flour dough fell within the range of approximately 0.88 g 100 g^{-1} of flour, whereas free lipids were found at a concentration of about 5.35 g 100 g^{-1} of flour.

Lipid translocation was reported in oat flour during processing (Angioloni & Collar, 2011). Specifically, lipids initially bound to proteins during dough mixing were subsequently observed to bind to starch during the baking process. Furthermore, the study reported that an increase in fibre content within the dough led to a reduction in lipid-protein and lipid-starch linkages due to interactions between fibres and endogenous biopolymers. Additionally, the presence of water was found to initiate lipid binding to the surface of protein granules, thereby diminishing the extractability of lipids by solvents (Angioloni & Collar, 2011).

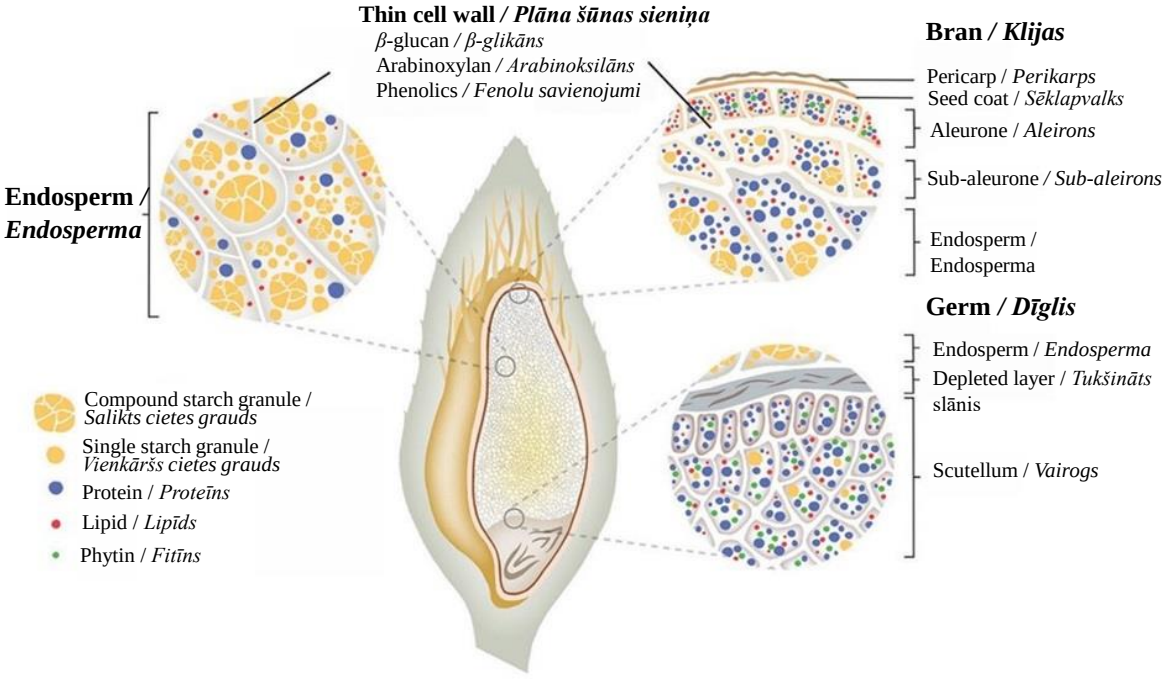


Figure 1.1. **Structural representation of the oat grain presenting oat tissues and the component distribution within these tissues (Grundy, Fardet, Tosh, Rich, & Wilde, 2018) /**

1.1. att. Auzu graudu strukturāls attēlojums, kas parāda auzu audus un komponentu sadalījumu šajos audos (Grundy et al., 2018)

Homogeneity in the proteins that bind lipids was observed between oat and wheat. The lipid-binding proteins isolated from the endosperms of oat and wheat displayed negligible differences in terms of amino acid content and molecular weight. The protein purification process involved the organic solvent extraction of oat and wheat endosperms, followed by HCl precipitation in a non-polar medium and preparative electrophoresis. The level of divergence observed between the purified proteins from oat and wheat fell within the range of variation observed in two different purification batches of the same protein (Ponz, Hernández-Lucas, Carbonero, & García-Olmedo, 1984).

It is evident that proteins interact with lipids through multiple mechanisms. Utilising phosphorus nuclear magnetic resonance spectroscopy and freeze-fracture electron microscopy, it was observed that lipids interact with wheat gluten through physical entrapment as well as polar and ionic bonding (McCann, Small, Batey, Wrigley, & Day, 2009).

When gluten was subjected to treatment with acetic acid (0.01-0.10*M*), alterations in the hydrophobicity and structural conformation of the protein were observed, leading to the binding of non-polar lipids. Concurrently, polar lipids were seen to interact with the protein through specific binding mechanisms. Furthermore, the dissociation of protein polymers coincided with the appearance of glycolipids, which exhibited further interactions with proteins through hydrophobic interactions and hydrogen bonding (McCann et al., 2009).

It is worth mentioning the existence of complexes between amylose and lipids, which can occur naturally in starch granules or be formed during crop processing (Chao et al., 2018; Le Bail et al., 1999). These complexes have a notable impact on various processing parameters, such as starch swelling power, gelatinisation temperature, gel rigidity, and even the reduction of starch susceptibility to enzymatic hydrolysis (Cai et al., 2021; Chao et al., 2018). Amylose–lipid complexes occur naturally, while amylopectin is known unlikely to form any complexes with lipids (Chen et al., 2021).

Protein and starch could not form complexes, due to being thermodynamically incompatible materials (Cai et al., 2021). Nevertheless, certain studies have indicated that the incompatibility of proteins depends on their classes (fractionation according to the Osborne classification) and their conformational states. This incompatibility can be enhanced through denaturation (Polyakov, Grinberg, & Tolstoguzov, 1997), as denaturation increases reactivity by exposing lysine residues that can bind to carbonyl groups (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016).

It has also been reported that native oat proteins have limited potential to interact with polysaccharides (Zhong et al., 2019), and they require modifications such as the aforementioned heat treatment to form conjugates. Conversely, increasing the protein ratio in the presence of polysaccharides, such as *β*-glucans, has not improved these conjugates, primarily due to steric hindrance caused by the polysaccharides (Zhong et al., 2019).

The reaction of the polysaccharides with protein including covalent bonding is discussed in Chapter 1.4.4, analysing protein modification.

Phytic acid

Certain minor grain components, such as phytates, exhibit reactivity and can form complexes with other grain compounds. Phytates, which are derivatives of phytic acid, are naturally present in fibre-rich plants like grains, whereas leafy vegetables and fruits contain only trace amounts of phytates (Ruican Wang & Guo, 2021). Phytic acid has 12 replaceable protons, with six of them being strongly acidic (pH 1.5–2.0), two weakly acidic (pH approximately 6.0), and the remaining 4 having pH values of 9.0 to 11.0. At a neutral pH, phytin, which carries a negative charge, can effectively bind cations such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+} and Ca^{2+} to form stable complexes (Angel, Tamim, Applegate, Dhandu, & Ellestad, 2002).

Other interactions involving phytic acid are also present. It is observable that phytin exhibits a high binding capacity with proteins and polysaccharides through electrostatic interactions (Ruican Wang & Guo, 2021).

At low pH, phytic acid binds to positively charged protein terminal groups and basic residues of amino acids, such as lysine or arginine. Metal-protein-phytate ternary complexes occur above the isoelectric point (Kaspchak, Mafra, & Mafra, 2018), typically forming via a cationic bridge involving Ca^{2+}(Ruican Wang & Guo, 2021).

The formation of binary phytate-protein complexes is influenced by factors such as pH, isoelectric point (p*I*), ionic strength, the availability of amino acids, and the presence of competitors. These formed protein-phytate complexes can significantly impact protein functionality. It has been suggested that phytates have a tendency to aggregate proteins, primarily through water displacement (Darby, Platts, Daniel, Cowieson, & Falconer, 2017), which subsequently can initiate protein precipitation or turbidity (Tran, Hatti-Kaul, Dalsgaard, & Yu, 2011).

Some globular proteins, such as soy glycinin, tend to bind phytates only at elevated temperatures (> 75 °C), as this initiates the denaturation process and exposes the charged groups of the protein (R. Wang, Liu, & Guo, 2018). The introduction of salts may significantly reduce the binding capacity of phytates (Kaspchak et al., 2018; R. Wang et al., 2018). Methods such as acid hydrolysis, autoclaving, and ion exchange can also reduce or completely degrade phytic acid. Nevertheless, the most effective means of phytate removal may involve enzymatic degradation by phytases (Handa, Sharma, Kaur, & Arya, 2020). However, it should be noted that releasing cations during phytate degradation could potentially destabilise proteins (Ruican Wang & Guo, 2021).

1.2.2. Protein extraction / *Proteīnu ekstrakcija*

The extraction and concentration of oat protein present a dilemma due to the presence of non-protein components that persist after the process. These unwanted components, which affect compositional and microstructural differences among protein ingredients, contribute to significant variations in nutritional performance and technical functionality within food systems (Loveday, 2020). For example, while fibre is generally a desirable component in certain applications like extrusion, it can pose limitations when solid particles are part of the protein source, such as in beverages.

In general, protein extraction and purification can be achieved through two primary methods: wet and dry extraction. Dry extraction or separation involves milling and sieving processes, while wet extraction relies on separation or precipitation technologies, each of which comes with its own set of challenges.

The following chapters delve deeper into oat protein extraction methods, providing an in-depth examination of both dry and liquid extraction processes. A significant focus is placed on the defatting process, given the relatively high oil content in oats, which requires its removal for effective protein purification.

Dry concentration

Dry fractionation of oats is generally considered more economically advantageous than wet fractionation because the latter requires significant energy input to dry the fractionated compounds. The process of purifying protein and separating it from starch is complicated by the small size of starch granules and the aggregates they form (Sibakov, 2014). Moreover, the soft groat characteristics and relatively high lipid content further complicate the fractionation process. During oat milling, the oats tend to adhere to the rolls, requiring the installation of cleaning devices. The incorporation of such a device, in conjunction with the utilisation of sieves with apertures larger than 212µm, facilitated the effective separation of bran from oat endosperm (R. Wang, Koutinas, & Campbell, 2007). However, processing oats with a lipid content exceeding 10.0% (dry basis) remains challenging, as sieves with apertures smaller than 212 µm, commonly used in wheat milling, become obstructed. Nonetheless, a proposed fractionation method has shown promise in achieving oat fractions containing up to 14.7% protein while being rich in dietary fibre (total dietary fibre content reaching up to 23.1%). Additionally, the starch content is reduced from an initial 63.7% to 49.7% using this method (R. Wang et al., 2007).

To obtain oat fractions with higher protein concentrations, a common practice involves an initial step of oat groat defatting. Kaukovirta-Norja (World Intellectual Property Organization Patent No. WO2008096044A1, 2008) developed a patented method for fractionating oats, where oat protein is separated as a by-product during the purification of oat beta-glucan. This method utilises a supercritical carbon dioxide system for lipid extraction, in certain examples in combination with ethanol. The defatted oat material then undergoes milling, sieving, and air separation processes. This fractionation method has the potential to yield specific fractions with protein contents as high as 78%. However, the exact protein concentrate yield from this process remains undisclosed.

Sibakov et al. (2011) obtained high-protein fractions through a process designed to yield oat fractions enriched in dietary fibre. According to their findings, oat fractions containing up to 73% protein were obtained after the third separation step. The removal of lipids was accomplished through the utilisation of supercritical CO_2 extraction. The methodology employed in the study was based on the distinctive attributes of the Hosokawa Alpine mill, featuring pin disc grinders. Subsequently, an air classification process was employed, with oat material being fed at a rate of 5 kg h^{-1} and an airflow set at 220±5 m^3 h^{-1}. These trials were conducted at a pilot scale, and the substantial amount (2310 kg) utilised in the trial suggests the potential for scaling up the process.

Moisio et al. (2015) reported similar findings using air classification to concentrate protein, achieving protein concentrations of up to 59% after removing oil through the supercritical CO_2 extraction from oat flour, followed by air classification. However, the specific yield of the oat protein concentrate was not disclosed. This method typically yields a protein fraction containing approximately 62.0% protein, 17.1% starch, 2.8% fat, and 2.0% dietary fibre (Jiang et al., 2015).

Similar results, applying air classification when concentrating protein, were reported by Moisio et al. (2015), who reached a protein concentration of up to 59% after oil removal by supercritical CO_2 from oat flour with subsequent air classification. The yield of the oat protein concentrate has not been disclosed. Such a method typically provides a protein fraction which contains 62.0% protein, 17.1% starch, 2.8% fat and 2.0% dietary fibre (Jiang et al., 2015).

Liquid extraction, concentration

Two distinct plant-based protein concentration approaches have been developed at the industrial level. These concentration methods rely on the protein structure of the raw material. Among these, soy and wheat can be classified as the most representative examples. For instance, soy covers over 65% of the global protein demand for plant-based protein. Soy isolate is typically produced by dissolving protein obtained from soybean meal at high pH, then supernatant after centrifugation passes precipitation at the isoelectric point, yielding at about 45% (Loman, Islam, Li, & Ju, 2016). The process of recovering wheat protein typically involves the physical separation of wheat starch from gluten particles in aqueous technological systems. This separation relies on the differences in solubility, particle size, and density between starch and protein materials (Sayaslan, 2004). This is possible due to the unique structure of wheat gluten. The wheat gluten in flour represents an irregular structure; however, in the presence of water, a three-dimensional network is formed. The formed layers are separated based on their density, wherein starch is the densest component. Then the gluten agglomerates pass through washing, dewatering and drying (Bergthaller, Witt, & Seiler, 2004; Cornell & Hoveling, 1998; Sayaslan, 2004; Sayaslan, Seib, & Chung, 2010).

The industrial process processing oats by separating starch, and subsequently protein, in its native form, is limited. The wet milling process generally used in starch industrial production is complicated, and unlike the wheat wet milling process, oat starch and protein cannot be separated by applying selective hydration and centrifugation. The protein and bran hydration level complicates the process (Saldivar, 2016). Moreover, the fibrous cellular structures within the endosperm of oats are composed of a mixed β-glucan, potentially blocking the efficient isolation of oat starch. Protein bound to starch and non-starch polysaccharides could not easily be extracted. Such a structure of oat starch requires a specific approach in protein purification. One of the representative examples is discussed below.

Reported attempts to isolate starch in its native form, treating oats at alkaline pH (with NaOH), resulted in protein content in tailings of up to 30% by weight (W. J. Lim, Liang, Seib, & Rao, 1992). Introducing enzymes such as proteases decreased the protein content in starch, although protein re-allocation during the extraction process was substantial. Protein after enzymatic solubilisation was then discharged as the supernatant fraction and tailing fraction. Protein concentration in dry matter reached up to 32.6%. The yield of recovered solubilised protein for the supernatant fraction was approximately at the level of 50% of the total protein in flour. The protein fraction which was recovered as tailings (top layer in pellet) in centrifugation had a protein concentration of about 25.9% in dry solids, whereas the yield was up to 22.8% of initial protein. The graphical representation of the oat wet milling process, which includes the isolation of starch and protein, is depicted in Figure 1.2. The introduction of proteases followed by cellulases in the aforementioned process did not yield any significant benefits. Attempting protein separation from starch by treating it with cellulases alone produced inferior results, with a substantial amount of protein remaining in the starch fraction (2.2–2.5%).

Solvent *extraction and precipitation*

In a study conducted by Yue and colleagues (2021), protein yields and structural characteristics were investigated using a choline chloride-dihydric alcohol deep eutectic solvent (DES) and its binary mixtures with water. The process involved subjecting oat flour to a DES solution in a ratio of 1:9, followed by heating to 80 °C for a duration ranging from 60 to 120 minutes. Subsequently, centrifugation was performed, and protein precipitation was carried out using DES. This method was acclaimed for its eco-friendliness due to its favourable attributes of biodegradability, low toxicity, and ease of application in food processing. The reported protein recovery rate was found to be dependent on both the extraction time and solution concentration, falling within the range of 10.5% to 42.9%. The corresponding protein concentrations ranged from 38.90% to 57.41%. The optimal extraction time was suggested to be 90 minutes at a temperature of 80 °C.

The solubilisation of oat protein in an alkaline environment followed by its subsequent precipitation is among the most commonly utilised methods for protein concentration. Ma (1983) isolated oat protein using alkaline and salt extraction methods. Both extracts demonstrated protein concentrations exceeding 90%. However, the alkaline extract had a significantly higher yield at 60%, while the salt extract yielded only 25%. Both isolates exhibited similar amino acid compositions, with a slightly higher lysine and total essential amino acid content observed in the alkaline isolate.

In the alkaline isolation method, the pH was adjusted to 9.5 using a diluted solution of NaOH (0.015 *N*) in a 1:8 ratio. Subsequently, the mixture was centrifuged, and the supernatant was neutralised, followed by a second centrifugation and freeze-drying. For the salt-based oat protein isolate, the process involved diluting the initial oat material with a 0.50 *M* $CaCl_2$ solution at a ratio of 1:10. This mixture was then subjected to centrifugation, dialysed against cold water, precipitated, centrifuged once more, and finally freeze-dried. This study closely aligns with prior research conducted by Cluskey et al. (1976), which revealed a wet extraction method for the production of oat protein concentrate. In their research, NaOH was employed to sustain the slurry at a pH of 9 during the extraction process.

Liu and colleagues (2009) isolated oat protein using an isoelectric precipitation method to explore the composition and secondary structure of oat protein. They employed an alkaline extraction method similar to that described by Ma (1983). The oat flour was mixed with water, and the pH was adjusted to 10.0 using a 2 *M* NaOH solution. After filtration through a mesh, the slurry underwent centrifugation at 3000 × g. The resulting supernatant was further centrifuged after pH adjustment to 5.0 using 0.50 *M* HCl. The resulting mixture underwent three washing cycles, followed by pH adjustment to 7.0 and subsequent freeze-drying. This process led to an oat protein concentration of 87.0%. In general, the amino acid composition was determined to be comparable to that of the initial oat material. However, the study reported an increase in the levels of isoleucine, methionine, phenylalanine, and arginine, while asparagine, serine, glycine, and cysteine were found to be present at lower concentrations in comparison to the initial raw material. The SDS-PAGE analysis revealed the presence of two predominant protein bands, with molecular weights of approximately 36 kDa and 22 kDa. These two peptide aggregates collectively accounted for 80% of the total protein content. Regarding the secondary structure of the oat protein isolate, it comprised approximately 74% β-sheet, 19% α-helix, and 7% β-turn structures. Additionally, it was observed that the oat protein concentrate exhibited a tendency to undergo self-assembly in aqueous solutions when the protein concentration exceeded 0.5 mg mL^{-1}. This property was suggested to enhance the stability of the protein in aqueous solutions by facilitating the formation of large aggregates (G. Liu et al., 2009). Unfortunately, the report did not include information regarding the yield of the oat protein isolate.

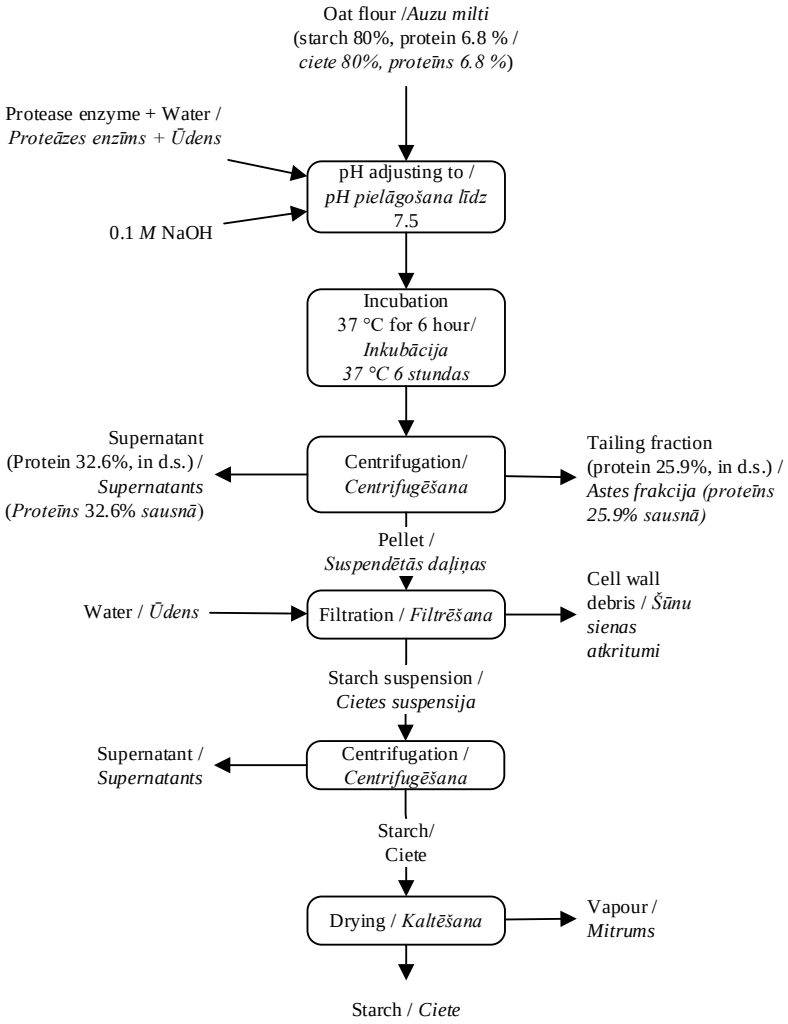


Figure 1.2. **Isolation of oat starch and protein in the wet milling process (adapted from W. J. Lim et al., 1992) /**
1.2. att. Auzu cietes un proteīnu izdalīšana mitrās malšanas procesā (pielāgots no W. J. Lim et al., 1992)

Ionic strength influence on protein precipitation

One potential approach to enhancing protein precipitation involves the consideration of the influence of salts, which can facilitate protein aggregation. Typically, salts have the capacity to induce protein aggregation. Globular proteins, for instance, have a tendency to increase in size at lower ionic strengths (0.001 *M* NaCl), forming small, elongated structures. These small structures subsequently lead to the development of larger aggregations when the ionic strength is increased to 0.1 *M*. It has been observed that these larger aggregations are formed through the random association of the previously developed smaller structures. (Durand, Gimel, & Nicolai, 2002). Furthermore, it was discovered that the aggregation process exhibited a significant reliance on temperature. It was hypothesised that the aggregation process induced by temperature was closely linked to protein denaturation. The requirement to elevate the ionic strength in order to promote the formation of larger aggregates could be attributed to the challenge of overcoming electrostatic repulsion (X. Li et al., 2009).

In a study conducted by X. Wang and colleagues (2018), a notable decline in zeta potential, which serves as an indicator of protein aggregation, was observed when soy protein isolate was exposed to varying concentrations of calcium sulphate. Specifically, this resulted in a reduction in zeta potential from -46.5 mV to -35.2 mV as the concentration of Ca^{2+} increased

from 0 to 10 mmol L^{-1}. This phenomenon was linked to the occurrence of protein aggregation within the soy emulsion.

The induction of gelation in soymilk under the influence of Ca^{2+} ions was also revealed, with slight alkaline conditions of up to pH 7.8 (Y. Li, Wan, Mamu, Liu, & Guo, 2022). The process of protein precipitation was found to be correlated with the concentration of calcium ions, reaching its maximum at 14 mmol L^{-1} for $CaCl_2$. The ions, particularly Ca^{2+} or Na^{+}, which carry a positive charge, function by neutralising the negative charge of the protein molecule through physical attraction.

The addition of NaCl increases the amount of Ca^{2+} required for protein precipitation, as the effect of Ca^{2+} is suppressed by NaCl (Yuan et al., 2002). It was reported that the addition of NaCl to the solution, up 0.3 *M,* increased protein solubility, contrary to the anticipated decrease in solubility due to the "salting out" effect. Parallel investigations indicated that elevating the salt concentration (up to 0.5 *M* NaCl) could lead to a subsequent increase in the solubility of the protein, particularly soy protein, in comparison to a concentration of 0.2 *M* NaCl. This observation remained consistent for both heated and unheated soy dispersions (Renkema, Gruppen, & van Vliet, 2002).

A similar impact of ionic strength was observed in the case of oat protein when salts, specifically NaCl and NaP, were introduced into the solution (R. Li & Xiong, 2021). Oat protein was extracted through alkaline solubilisation, followed by subsequent precipitation at the isoelectric point. Figure 1.3 illustrates the development of protein aggregates at various pH levels and NaCl salt concentrations in an aqueous solution.

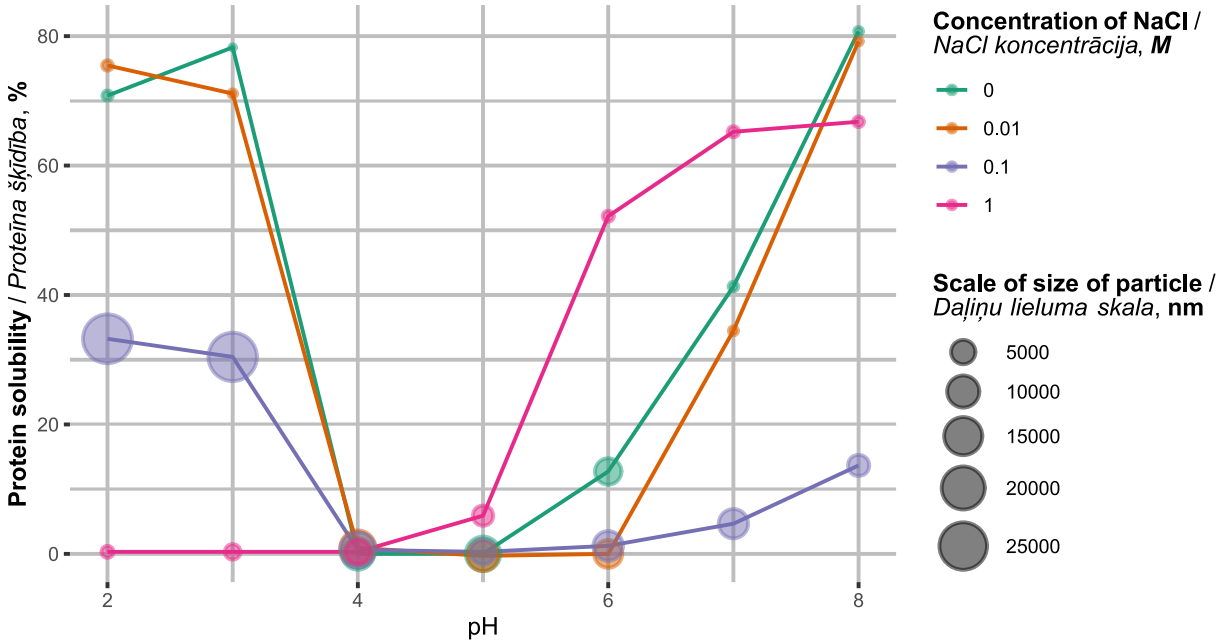


Figure 1.3. **NaCl salt influence on oat protein particle size development and solubility (amended from R. Li & Xiong, 2021) /**
1.3. att. NaCl sāls ietekme uz auzu proteīna daļiņu izmēru veidošanos un šķīdību (pārveidots no R. Li & Xiong, 2021)

Under neutral pH conditions, protein solubility demonstrated a declining trend and reached a plateau at approximately 0.1 *M* NaCl concentration. Simultaneously, the increase in salt concentration led to an elevation in protein solubility under neutral pH conditions. The reported findings on particle size development unveiled a robust negative correlation with the pH-solubility curve when salt concentrations were either low (0.01 *M* NaCl), absent, or high (1 *M* NaCl). However, at a salt concentration of 0.1 *M*, the particle size development exhibited distinct behaviour. It reached its maximum at pH 2 (> 26 000 nm) and subsequently decreased at pH 5 (> 5 000 nm).

Interestingly, the solubility of the protein did not exhibit a correlation with particle size at 0.1 *M* NaCl. While the protein remained relatively soluble under harsh acidic conditions (pH 2), the size of the protein particles grew to extremely large dimensions. It was speculated that the observed phenomenon might be related to the stronger interaction between Na^+ and COO^- as compared to $NH_3^+$and Cl^-, which contributed to the size-solubility relationship.

Oat protein solubility does not appear to be correlated with particle size; instead, it should be considered a function dependent on ionic strength and pH. It could be assumed that particle size might facilitate the prediction of protein separation techniques.

Further discussion involves a protein isolation method based on enzymatic extraction.

Enzymatic extraction

Protein concentration involves releasing protein from accompanying materials, thereby reducing their presence in the final product, particularly starch, which comprises up to 60% (Zhu, 2017) in raw oats. Enzymatic breakdown of starch chains generally involves 4 types of enzymes. Amylases hydrolyse (1,4) α-D-glucosidic bonds, isoamylases hydrolyse (1,6) α-D-glucosidic bonds, glucanosyltransferases transfer (1,4) α-D-glucosidic bonds and starch branching enzymes transferases catalysing the hydrolysis of the α-(1,4)-linked linear chains of amylose and amylopectin promoting the formation of new α -(1,6) linked branch chains (Yang Li et al., 2018; Robyt, 2009). Furthermore, amylases are divided into three classes–endo-acting α-amylases, exo-acting and β-amylases, and isoamylases (Robyt, 2009). The former two enzyme groups are employed for breaking down starch into smaller fragments during starch hydrolysis which might pass 3 steps: gelatinisation, liquefaction, and saccharification. Figure 1.4. demonstrates enzymes involved in starch degradation. While the gelatinisation phase is required to increase the accessibility of the substrate, in the liquefaction phase, the gelatinised starch undergoes partial hydrolysis, resulting in a product with a dextrose equivalent ranging from 15 to 30. During saccharification, the dextrose equivalent range increases from 40 to 90 (Baks, Bruins, Matser, Janssen, & Boom, 2008).

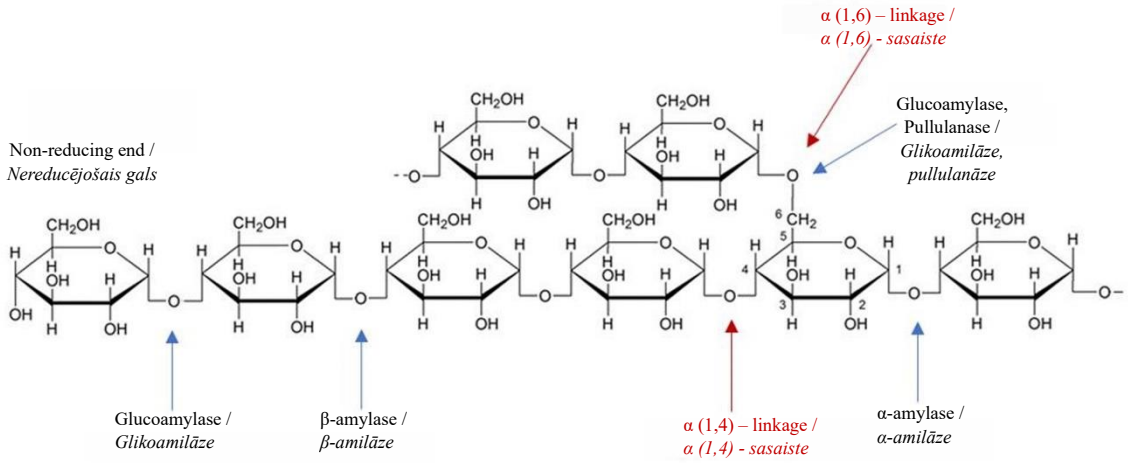


Figure 1.4. **Enzymes involved in starch degradation /**
1.4. att. Fermenti, kas iesaistīti cietes noārdīšanā

Enzymes preserving the α-glycosidic bond configuration follow the classical bimolecular nucleophilic substitution mechanism presented in Figure 1.5. The initiation of the bond cleavage in the glucopyranosyl unit undergoing hydrolysis is catalysed by the carboxylate base through a nucleophilic attack at C-1, wherein aspartate acts as a nucleophile (Haren, 2002; S. J. Lim & Oslan, 2021). Concurrently, the carboxylic acid group (glutamic acid in acid form as a source) displaces and protonates the glycosidic oxygen. The resultant carboxylate group in attack forms a covalent β-linked acetal-ester, leading to the formation of a glucopyranosyl-

enzyme intermediate. This high-energy linkage is subsequently hydrolysed by water, with the second aspartate carboxylate group at the enzyme's active site facilitating the reaction. The carboxylate group extracts a proton from water, enhancing its nucleophilicity for an attack on the β-linked acetal-ester. As a consequence, the anomeric carbon atom of the released glucopyranosyl unit from the enzyme complex assumes an α-configuration, ensuring the retention of the product's configuration at its reducing end (Robyt, 2009).

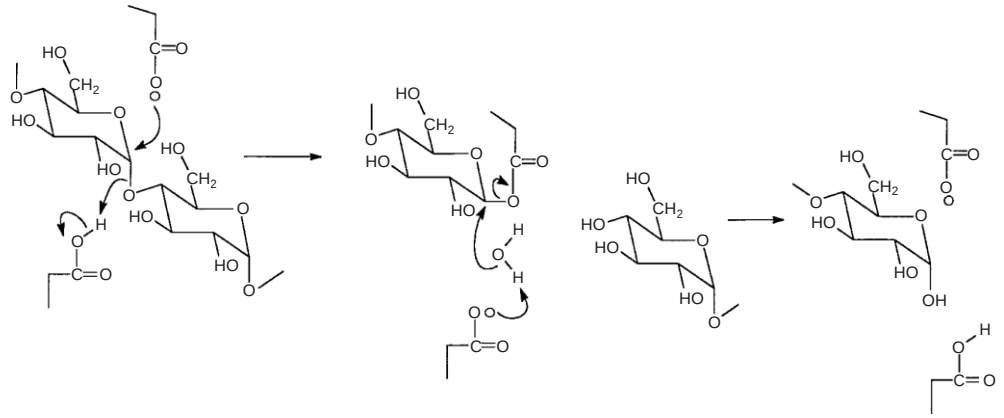


Figure 1.5. **Mechanism of the hydrolysis of glycosidic linkages (adapted from Robyt, 2009) /**
1.5. att. Glikozīdu saišu hidrolīzes mehānisms (pielāgots no Robyt, 2009)

A similar mechanism represents the breakdown of the α-(1,6) branch linkage through isoamylases, as well as for cyclomaltodextrin glucanosyltransferase. In the case of the latter enzyme, the water molecule is substituted by the C-4 hydroxyl group located on the non-reducing end glucosyl unit within the starch chain (Robyt, 2009).

Enzyme treatment is also considered for non-starch polysaccharides, as the presence of non-starch polysaccharides in oats might significantly affect the technological process due to increased viscosity, altering the rheological behaviour in aqueous media (Heidary Vinche, Khanahmadi, Ataei, & Danafar, 2021; Heidary Vinche et al., 2021; Yan, Yang, Jiang, Liu, & Yang, 2018) or binding protein in matrix (Aiello et al., 2021). Arabinoxylans and β-glucans comprise the major part of soluble non-starch polysaccharides (Barjuan Grau, Vishaal Mohan, Tovar, & Zambrano, 2023; Sargautiene, Nakurte, & Nikolajeva, 2018). β-glucans, more specifically (1,3), (1,4)-β-D-glucan, might effectively be degraded by β-1,3-1,4-glucanases which belong to a category of hydrolytic enzymes that facilitate the breakdown of β-1,4-glycosidic bonds situated next to 3-O-substituted glucose residues in the structure of mixed-linked β-glucans (Yan et al., 2018). The typical structure of arabinoxylans comprises a linear sequence of (1,4)-β-α-xylopyranoside (Xylp) units, featuring α-L-arabinofuranosyl substitutions linked via α-(1,2) and/or α-(1,3) glycosidic bonds. As a result, Xylp residues with mono- and/or di-substitutions at the O-2 and O-3 positions, along with unsubstituted residues, may be present (Barjuan Grau et al., 2023). The primary enzymes responsible for the degradation of arabinoxylans are xylanases (J. Wang et al., 2020). The standard guideline for depolymerising xylanase is its ability to efficiently cleave the β-(1,4)-linkages between xylopyranoside residues in xylans through hydrolysis (Beaugrand et al., 2004). It has been reported that xylanases induce the co-solubilisation of β-glucan, an outcome arising from the disassembly of the cell wall (Beaugrand et al., 2004).

Multiple studies have demonstrated the application of enzymes in the process of extracting oat proteins. Prosekov et al. (2018) introduced a method for isolating oat proteins specifically from oat brans. Defatted oat brans were subjected to treatment with the amyloglucosidase enzyme. The breakdown of the cell wall polysaccharide membrane was expected to lead to the release of protein into the suspension, which was subsequently separated. The suspended solids were washed to create a protein-rich fraction with a concentration of up

to 83.8% (determined by the Dumas method). Although the yield was not reported, the functional properties of the obtained protein were found to be improved compared to alkali extraction methods, as discussed in the subsequent chapters.

Another study demonstrated a combined enzymatic and alkaline method for extracting oat protein from oat brans (Jodayree, Smith, & Tsopmo, 2012). Oat protein extraction involves the use of various enzymes, specifically with the primary enzymatic activities being xylanase, α-amylase, amyloglucosidase, and cellulase. Subsequently, the slurries were subjected to a pH adjustment to 9.5 using 2 *M* NaOH and then centrifuged. The resulting supernatant was collected and precipitated. The highest protein concentration observed, reaching 82% (determined by the modified Lowry method), was found in the sample treated with amyloglucosidase. Following this, the obtained protein isolates were subjected to endo-protease treatment to enhance their antioxidative properties.

An effort to enhance protein extraction prior to alkaline extraction (at a pH 9.5) was undertaken by subjecting oat brans to enzymatic treatment using an enzyme with declared β-glucanase activity, which aimed to break down non-starch polysaccharides. The enzymatic pre-treatment resulted in significantly higher protein recovery, reaching 56%, compared to alkaline precipitation without enzymatic pre-treatment, which yielded only 15% of the total protein (Guan & Yao, 2008).

Immonen et al. (2021) introduced a modified enzymatic extraction method that involved protein solubilisation using protein-glutaminase, followed by protein concentration through ultrafiltration. Prior to introducing protein-glutaminase, starch solubilisation was achieved using various carbohydrates. Deamidation resulted in improved protein concentration after ultrafiltration, increasing it from 45.0% to 52.4% compared to the protein concentrate that was not treated with protein-glutaminase. Both oat protein concentrates exhibited significantly enhanced solubility at neutral and slightly alkaline pH levels compared to the solubility of proteins extracted from the initial raw material.

Protein extraction from oat press cake, an industrial by-product in oat drink production, was recently investigated by Aiello et al. (2021). Oat cake underwent treatment with various enzymes, either individually or in combinations, including α-amylase, a mixture of cellulase and xylanase, and protease. Following treatment, the samples were subjected to centrifugation, and the protein was precipitated from the supernatant by adjusting the pH to 5.0 using 0.1 *M* HCl. This was then followed by centrifugation and drying. SDS-PAGE analysis revealed the presence of proteins at 10 kDa and 17 kDa, whereas the initial protein samples exhibited bands within the range of 22–24 kDa and 32–35 kDa. The protein content in the initial oat cake was 32.4% by weight, although the yield and concentration of the obtained protein samples were not reported.

Summarised oat protein extraction methods

Table 1.1 provides a summary of the key findings from the most relevant literature discussed above. The studies explored various oat protein extraction methods to yield protein concentrates, outlining both the purpose of the research and the characteristics of the proteins.

Table 1.1. / *1.1. tabula*

Oat protein extraction methods described in different studies */ Dažādos pētījumos aprakstītas auzu proteīna ekstrakcijas metodes*

Method characteristics / *Metožu raksturojums*	**Purpose of research /** *Pētījuma mērķis*	**Protein characteristics /** *Proteīnu īpašības*	**Source /** *Avots*
1	**2**	**3**	**4**
Air separation / *Aeroseparācija*	β-glucan / *β-glikāns*	Concentration / *Koncentrācija* 78%	(World Intellectual Property Organization Patent No. WO2008096044A1, 2008)
Air separation / *Aeroseparācija*	β-glucan / *β-glikāns*	Concentration / *Koncentrācija* 73%	(Sibakov, 2014)
Air separation / *Aeroseparācija*	Protein for extrusion / *Proteīns ekstrūzijai*	Concentration / *Koncentrācija* 78%	(Moisio et al., 2015)
Roller milling, sifting / *Malšana, sijāšana*	Fractioning excluding defatting / *Frakcionēšana, izņemot attaukošanu*	Concentration / *Koncentrācija* 14.7%	(R. Wang et al., 2007)
Wet milling, alkaline extraction with subsequent protein solubilisation by enzymes / *Mitrā malšana, sārma ekstrakcija ar proteīnu izšķīdināšanu ar fermentiem*	Native starch / *Ciete*	Concentration / *Koncentrācija* 32.6%	(W. J. Lim et al., 1992)
Solvent extraction, choline chloride-dihydric alcohol precipitation / *Ekstrakcija ar šķīdinātāju, holīna hlorīda-dihidrospirta izgulsnēšana*	Protein recovery / *Proteīnu ieguve*	Recovery up to 42.9%, concentration up to 57.4% / *Ieguve līdz 42,9%, koncentrācija līdz 57,4%*	(Yue, Zhu, et al., 2021)
Alkaline extraction by NaOH, pH 9.5 / *Sārma ekstrakcija ar NaOH, pH 9,5*	Protein recovery, protein functionality / *Proteīnu ieguve, proteīnu funkcionalitāte*	Concentration 90%, yield 60% / *Koncentrācija 90%, iznākums 60%*	(C. Y. Ma, 1983)
Salt extraction, by 0.5 *M* $CaCl_2$ / *Sāls ekstrakcija ar 0,5 M* $CaCl_2$	Protein recovery, protein functionality / *Proteīnu ieguve, proteīnu funkcionalitāte*	Concentration 90%, yield 25% / *Koncentrācija 90%, iznākums 25%*	(C. Y. Ma, 1983)
Alkaline extraction by 10 *M* NaOH, pH 9.0 / *Sārma ekstrakcija ar 10 M NaOH, pH 9,0*	Protein recovery / *Proteīnu ieguve*	Concentration 76%, yield 22% / *Koncentrācija 76%, iznākums 22%*	(Cluskey et al., 1976)

Continuation of Table 1.1. / *1.1. tabulas turpinājums*

1	2	3	4
Alkaline extraction by 2 *M* NaOH, pH 10, filtrated, centrifuged / *Sārma ekstrakcija ar 2 M NaOH, pH 10, filtrēts, centrifugēts*	Protein recovery, structure identification / *Proteīnu ieguve, struktūras noteikšana*	Concentration 78% / *Koncentrācija 78%*	(G. Liu et al., 2009)
Alkaline extraction at pH 10 with following salt induced protein aggregation / *Sārma ekstrakcija pie pH 10 ar sekojošu sāls izraisītu proteīnu agregāciju*	Protein solubility / *Proteīnu šķīdība*	Information is not available / *Informācija nav pieejama*	(R. Li & Xiong, 2021)
Enzymatic, treating oat brans with glucoamylase / *Fermentatīva, apstrādā auzu klijas ar glikoamilāzi*	Protein functionality / *Proteīnu funkcionalitāte*	Concentration 83% *Koncentrācija 83%*	(Prosekov et al., 2018)
Alkaline extraction followed by enzymatic pre-treatment with various carbohydrases / *Sārma ekstrakcija, kam seko fermentatīvā pirmapstrāde ar dažādām ogļhidrāzēm*	Protein recovery, peptide identification / *Proteīnu ieguve, peptīdu identificēšana*	Concentration up to 82% / *Koncentrācija līdz 82%*	(Jodayree et al., 2012)
Alkaline extraction followed by enzymatic pre-treatment with β-glucanase / *Sārmu ekstrakcija, kam seko fermentatīva pirmapstrāde ar β-glikanāzi*	Protein recovery / *Proteīnu ieguve*	Concentration up to 82%, yield 56% / *Koncentrācija līdz 82%, iznākums 56%*	(Guan & Yao, 2008)
Enzymatic extraction treating with carbohydrases, with subsequent protein enzymatic solubilisation and recovery through ultrafiltration / *Enzimātiskā ekstrakcija, apstrāde ar ogļhidrāzēm, pēc tam proteīnu fermentatīvā šķīdināšana un atgūšana ar ultrafiltrācijas palīdzību*	Protein recovery, protein functionality / *Proteīnu ieguve, proteīnu funkcionalitāte*	Concentration up to 50% / *Koncentrācija līdz 50%*	(Immonen, Myllyviita, et al., 2021)
Enzymatic protein extraction from insoluble by-product by various enzymes, recovery through precipitation / *Enzīmātiskā proteīnu ekstrakcija ar dažādiem enzīmiem no nešķīstošiem blakusproduktiem, atgūšana ar nogulsnēm*	Protein structure, bounded phenols, peptides / *Proteīnu struktūra, saistītie fenoli, peptīdi*	Information is not available / *Informācija nav pieejama*	(Aiello et al., 2021)

1.2.3. Protein defatting / *Proteīnu attaukošana*

Oats are characterised by a substantial lipid content, influenced by factors such as oat variety and cultivation conditions. Kouřimská et al. (2018) conducted an investigation on oat varieties and found oil content ranging from 4.3% to 6.1%. Similarly, Leonova et al. (2008) reported oil content spanning from 7.2% to 8.5%. In contrast, Sterna et al. (2016) documented a wider range of oil content in oats, extending in certain samples from 5.2% to 12.4% by weight. Notably, naked oats exhibited a higher average oil content of 9.7%, compared to 5.2% in husked oats. Oat selections with elevated oil content can achieve even higher concentrations. Researchers at Iowa State University managed to obtain oil concentrations in oats of up to 18.1% through recurrent selection breeding programmes (Peterson & Wood, 1997).

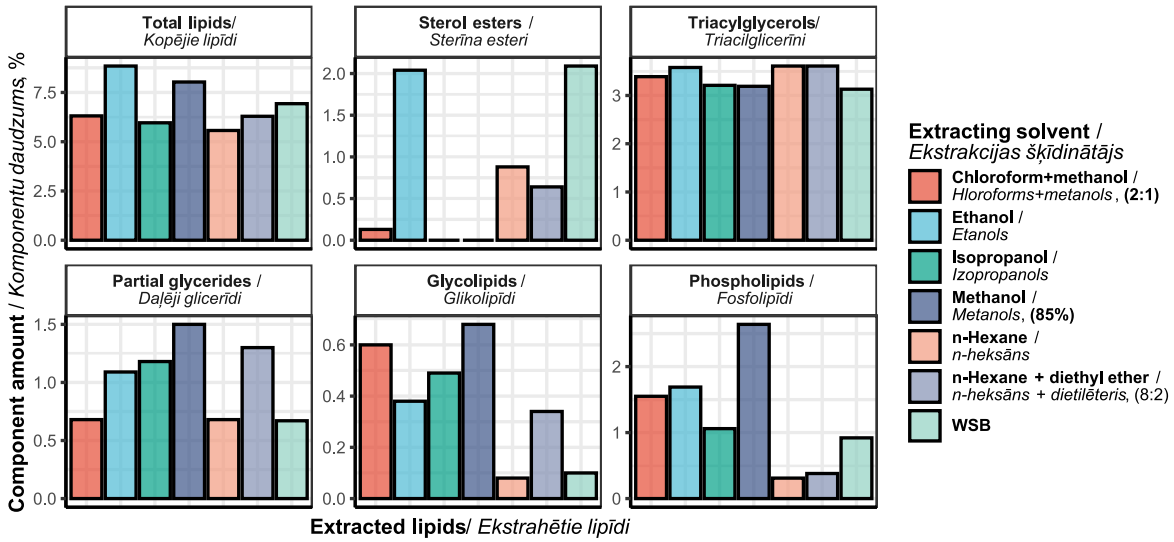
Relatively high oil content in the raw oat material causes challenges during oat processing, as briefly addressed in the section on dry fractionation. Furthermore, lipid content could substantially increase in protein fraction during the purification process, requiring a subsequent technological step of defatting.

The following paragraphs update data on methods applied for material defatting, including a review of process parameters and specific properties of solvents and pre-treatment methods facilitating the defatting process; special attention is paid to processes involved in crop defatting and the defatting effect on protein properties. Two major types of defatting methods are presented in more detail–lipid removal using ethanol and supercritical CO_2 extraction.

Solvent extraction

In lipid extraction processes from plants, mechanical pressing followed by solvent extraction is a common practice, with commercial hexane being the widely preferred non-polar solvent. However, hexane's persistent health concerns and classification as a highly toxic air pollutant raise significant issues (Capellini, Chiavoloni, Giacomini, & Rodrigues, 2019; De Pretto, Tardioli, & Costa, 2017). Oat lipids are higher in other components than triglycerides compared to common oil seeds like soy, wherein hexane is typically employed. Various lipid solvent extraction systems could be considered for the sufficient defatting of oat fractions, depending on the targeted lipid component. Oat defatting could be performed by single polar or non-polar systems, as well as combinations of these, such as water-saturated n-butanol (M. Zhou et al., 1999), enabling the selective extraction of the lipids. Lipid extraction efficiency and composition substantially depend on the type of solvent chosen. Hexane-based systems or ether demonstrate high efficiency in extracting non-polar lipids. However, when extracting polar cereal lipids, the efficiency of non-polar solvents sharply drops, in particular with phospholipids, which are associated with crop cell membranes. Short-chain alcoholic solvents such as ethanol and isopropanol might yield a higher content of phospholipids and unsaponifiable material than hexane (Capellini, Giacomini, Cuevas, & Rodrigues, 2017; Nagendra, Sanjay, Khatokar, Vismaya, & Nanjunda, 2011). The polar solvents break hydrogen bonds and electrostatic forces binding the phosphate group to the lipid group, thereby facilitating the transfer of these into the solvent.

The efficiency of multiple solvent extraction systems in oats has been reported (Sahasrabudhe, 1979; M. Zhou et al., 1999). As shown in Figure 1.6. all solvents extracted most of the triglycerides, which ranged from 3.13 to 3.61%. Ethanol and water-saturated n-butanol extracted more sterol esters compared with other employed solvents. Diethyl ether and alcohols extracted more partial glycerides. The amount of total lipids extracted varied from 5.6% to 8.8%, while the most efficient solvent was identified as ethanol.



WSB – water-saturated n-butanol / *WSB – ar ūdeni piesātināts n-butanols*

Figure 1.6. **Composition and amount of lipids extracted from oat groats by different solvents, % of mass (modified from M. Zhou et al., 1999) /**
1.6. att. No auzu putraimiem ar dažādiem šķīdinātājiem ekstrahēto lipīdu sastāvs un daudzums, masas % (modificēts no Zhou et al., 1999)

Ethanol also excels when considering technological aspects. Due to the lower miscibility at room temperature or below, where oil solubility is less than 1%, ethanol is easier to recover than hexane; the two phases after high-temperature extraction with subsequent cooling of the extract result in an alcohol and oil-rich phase (Oliveira, Garavazo, & Rodrigues, 2012). This excludes the complicated technological step of oil desolventization, wherein the solvent should be removed by evaporation, with subsequent solvent recovery through distillation. Applying such a technique could reduce the consumption of energy by up to 30% compared to other non-polar solvents such as hexane (Johnson & Lusas, 1983). Another rationale for opting for ethanol as a solvent is its growing recognition within the food industry, particularly in the context of health-conscious products, as an alternative and renewable solvent (Potrich et al., 2020).

The purity of alcohol plays a crucial role in the capacity of lipid extraction, as water, a polar solvent, diminishes oil extractability (Capellini et al., 2017). Nevertheless, it is essential to emphasise that the purity of ethanol also has a direct impact on the extractability of non-lipid components, which are typically present in the material, such as sugars and phosphatides, which have been demonstrated to be effectively extracted using water (Johnson & Lusas, 1983; Navarro, Capellini, Aracava, & Rodrigues, 2016). Additionally, there is a positive correlation between temperature and the efficiency of lipid extraction using ethanol. Elevated temperatures, specifically within the range of 60–90 °C, substantially enhance the lipid extraction process. (Navarro et al., 2016; Sawada, Venâncio, Toda, & Rodrigues, 2014).

Material pre-treatment may enhance lipid extraction. Nagendra et al. (2011) observed that enzymatic treatment using cellulase and pectinase had a positive effect on oil yield from rice bran when this enzymatic treatment was conducted prior to solvent extraction.

Protein transfer into solvent

When applying solvent for oil extraction, it is essential to consider the extraction of proteins and other extractable components. The loss of protein or its redistribution between the extract and raffinate phases depends on both the treatment conditions and the protein composition of the solute. Sawada et al. (2014) conducted a trial where they observed protein content loss when soybeans were treated with ethanol at concentrations ranging from absolute

ethanol to 6% and 12% water/ethanol mixtures. These processes were aimed at extracting oil from soybeans. Alongside oil extraction, a significant loss of protein was observed when soybean meal was treated at temperatures ranging from 40 to 90 °C. The most substantial protein transfer to the extract phase was noted at the lowest temperature and the highest water concentration in the water/ethanol mixture (12%). Interestingly, increased temperature reduced protein transfer to the extract phase, with protein loss decreasing to about 2% at 90 °C compared to approximately 7% at 40 °C. The protein transfer rate was expressed as g 100 g^{-1} soybean (wet basis).

Similar results were reported by Kwiatkowski and Cheryan (2002), who investigated oil and protein extraction using water/ethanol mixtures. The authors concluded that ethanol extraction capacity was highest at 70% ethanol and lowest at absolute ethanol. It was suggested that most of the extracted protein was zein, a hydrophobic alcohol-soluble protein constituting approximately 40% of the total protein in corn. The amount of protein extracted ranged from less than 0.5% to up to 2%, using absolute and 70% ethanol, respectively. Protein extraction capacity was determined on g $100g^{-1}$ corn, with the initial protein content averaging 8.2% on a wet basis.

Supercritical carbon dioxide extraction

Oil extraction using supercritical carbon dioxide (CO_2) can be considered as an alternative to solvent extraction. This process is relatively new, with more research attention being drawn to it over the past two decades.

Several advantages have been identified regarding the use of supercritical fluids in the industry, including environmental benefits, improvements in health and safety, and the versatility of the process itself. Knez et al. (2010) described supercritical fluids as having a less damaging impact on environmental issues compared to conventional organic solvents. However, limited information was provided regarding the methodology supporting this statement. One of the major advantages related to process applicability is associated with the favourable thermophysical properties of supercritical fluids. These properties include low viscosity, low density, high diffusion, and a high dielectric constant of the system, which can be easily tailored by controlling temperature and pressure parameters.

Gases under supercritical fluid conditions possess high solubilising capacity, exhibiting properties intrinsic to both liquids and vapours (Rad, Sabet, & Varaminian, 2019). Carbon dioxide is a commonly chosen supercritical fluid due to its advantages, including low toxicity, nonflammability, and stability in thermodynamic systems (Knez, 2016). Its linear structure ensures good permeability (Rad et al., 2019). The solubility of gases in rapeseed oil was studied, revealing that carbon dioxide is one of the most soluble gases, with short-chain hydrocarbons such as acetylene being even more soluble, particularly acetylene (Korobeynikov & Anikeeva, 2016; Swidersky & Guo, 2008).

Interestingly, increasing the temperature from 20 °C to 40 °C reduced the solubility of gases, with the exception of oxygen. This decrease in solubility is a typical outcome when temperature rises at constant pressure, as it leads to a decrease in the density of the solvent, a condition that usually decreases the solubility of the solute. Simultaneously, an increase in temperature raises the vapour pressure of the solute, which might enhance the solute's solubility in the system (Hrnčič, Cör, Verboten, & Knez, 2018).

Rai et al. (2016) investigated how the yield correlates with temperature during supercritical extraction using carbon dioxide on sunflower oil. They concluded that temperature positively affects yield. They speculated that this effect is achieved due to an increase in the mass transfer coefficient, as the stimulation of diffusivity of sunflower oil in carbon dioxide is increased. In parallel, the solute's mass transfer rate also increases at higher temperatures. Generally, the temperature range used for seed oil extraction falls within the range of 40 °C to 80 °C (Ahangari, King, Ehsani, & Yousefi, 2021).

Pressure is a key factor in supercritical fluid extraction, exerting a more direct influence on solute than temperature. Increasing pressure enhances solubility, likely due to the higher density of the medium (Hrnčič et al., 2018). However, research indicates that the impact of pressure on recovering minor oil compounds (tocopherols, sterols, and sterol esters) varies for each compound, as demonstrated in sunflower deodoriser distillates (Vázquez et al., 2006). Notably, solution enrichment generally rises with increased pressure. It was emphasised that raw material selection is critical in determining extraction conditions. Typically, the pressure range used for supercritical extraction of seed oils in various studies falls within the range of 10 mPa to 35 mPa (Ahangari et al., 2021).

Several limitations associated with using carbon dioxide as a supercritical fluid should be taken into consideration. These limitations include solvent polarity and a low capacity to form interactions with solutes. Carbon dioxide, being a nonpolar solvent, requires the use of additives or co-solvents to alter its polarity. This modification consequently enhances its solubility and interactivity with solutes (Behjati, Karimi, & Varaminian, 2019).

The extensive use of supercritical fluid processes has been impeded by the substantial capital and operational expenses associated with the required equipment. Generally, the products treated using supercritical gases tend to have higher costs compared to conventional applications (Knez, 2016). The cost per tonne of raw material depends significantly on the system's design and may reach up to € 60 kg^{-1} for small batch capacities. However, large-scale counterflow systems could be established at a cost as low as € 0.06 kg^{-1} (Hrnčič et al., 2018). Many studies have investigated supercritical extraction, typically using carbon dioxide as a solvent. The main groups of compounds subjected to treatment include essential oils, phenolic compounds, lipids, carotenoids, and alkaloids (W. Wang, Rao, et al., 2021). The most prominent applications of supercritical fluid extraction in industry primarily involve the extraction of hop compounds or the decaffeination of coffee or tea. Installations for spices used in food and natural cosmetic ingredients operate on a smaller scale (Knez, 2016). In most of the reviewed systems, carbon dioxide is used as a solvent, often with the introduction of co-solvents, particularly ethanol.

Defatting of oats by SC-CO_2

The extraction of lipids from plant seeds using supercritical fluid extraction has been widely employed (W. Wang, Rao, et al., 2021), including oats and their constituents. Aro et al. (2007) conducted an oat lipid extraction study from oat groats and flakes using supercritical extraction at 450 bar and 70 °C. In certain trials, ethanol was employed as a co-solvent with carbon dioxide as the extraction fluid. The extraction of crude oil yielded approximately 87% during the two-step extraction process, both with carbon dioxide alone and with the addition of ethanol as a co-solvent. It is noteworthy that oat flakes exhibited a 1.7-fold higher oil yield compared to groats.

Another research study was conducted by Fernández-Acosta et al. (2019) to evaluate the influence of various independent variables, such as pressure, temperature, particle size, and others, on the yield of oat crude oil and fatty acids in a supercritical fluid system where carbon dioxide was utilised as the supercritical fluid. The highest yield of crude oat oil was achieved at the highest tested pressure of 55 mPa when the particle size exceeded 250 μm. The yield reached approximately 54% when compared to the Soxhlet extraction method. However, for specific fatty acids, especially linoleic acid, pressure was not the most significant variable; instead, particle size emerged as the most crucial factor.

Similarly, another report indicated that particle size was the second most significant factor influencing oil extraction, followed by pre-treatment (wherein raw material was treated with 1 *M* NaOH to release bound fatty acids or polyphenols), and temperature, while other variables exhibited low significance. It is worth noting that in a reported study, the extraction of lipids from milled oat groats, followed by SC-CO_2 extraction, primarily targeted non-polar lipids (Kaukonen et al., 2011).

Various solvents and supercritical fluid systems have been utilised to extract different oat components. Lipids and phospholipids were extracted from oats using propane or dimethyl ether (Y. Li et al., 2021), and butane was employed to extract oil from oat brans (Guan, Jin, Li, Huang, & Liu, 2018). Additionally, supercritical fluid systems have been used to extract various polyphenols from oats (Walters, Udenigwe, & Tsopmo, 2018), with some studies employing SC-CO_2 and ethanol as a co-solvent (Escobedo-Flores, Chavez-Flores, Salmeron, Molina-Guerrero, & Perez-Vega, 2018). Aroma volatiles in oat flakes have also been extracted using SC-CO_2 (Morello, 1993). Furthermore, some reports have investigated changes in oat compounds, such as starch, as well as thermal transitions in starch gelatinisation and amylose-lipid complexes. These studies have also characterised various functional properties of oat compounds following supercritical fluid extraction (Stevenson, Eller, Radosavljević, Jane, & Inglett, 2007).

1.3. Principal functional properties of oat protein / *Auzu proteīna galvenās funkcionālās īpašības*

Functional properties of proteins encompass their physicochemical characteristics that have a significant impact on how they interact within food systems across various stages, including preparation, processing, storage, and consumption. These properties play a crucial role in shaping the quality and sensory attributes of food products. In the context of food applications, Kinsela (1976) and, later, Zayas (1997b) have classified the primary functional properties of proteins as follows: a) hydrophilic properties, related to aspects such as protein solubility, swelling behaviour, water holding capacity, foaming capabilities, and gelling capacity; b) hydrophilic-hydrophobic properties, including properties related to emulsification and foaming, indicating their ability to interact with both water-based and oil-based components; c) hydrophobic properties which specifically involve the binding capacity of proteins to fats.

These functional characteristics are fundamental in determining how proteins behave within food systems and, consequently, influence the overall attributes and quality of food products. The aspects related to oat protein, to some degree, are discussed further in the following sections.

1.3.1. Protein solubility / *Proteīnu šķīdība*

The solubility of oat protein is significantly influenced by the pH. Under alkaline methods, minimal solubility is observed at pH 5, while under salt-based methods, minimal solubility occurs at pH 6. Despite the structural similarity of oat globulins to the 11S globulins found in legumes, oat proteins demonstrate lower solubility in salt-based solutions (Brinegar & Peterson, 1982). Loponen et al. (2007) conducted an investigation into the solubility of oat globulins isolated from oat brans, with a focus on their behaviour under lactic acid fermentation conditions while controlling pH and salt concentration. In solutions with 1 *M* NaCl and 0.5 *M* NaCl, the protein demonstrated satisfactory solubility at a pH ranging from 7 to 8. However, at a pH of 5 and below, the protein became insoluble at these salt concentrations. In contrast, in low-salt conditions (0.05 *M* NaCl), the protein remained soluble even under acidic conditions. Notably, solubility began to increase significantly at a pH of 4 and below. Loponen et al. (2007) hypothesised that the acidic conditions present during lactic acid fermentation might lead to protein unfolding, resulting in the formation of globulin aggregates that subsequently reduce protein solubility in salt-buffer solutions. However, an alternative explanation for protein solubility in low or non-salt solutions was not provided.

Prosekov et al. (2018) reported a substantial increase in the solubility of oat protein following enzymatic extraction using amyloglucosidase. Surprisingly, the highest solubility of oat protein was attained within the pH range of 5–6, resulting in a nitrogen solubility index of

approximately 50%. This level of solubility represented a significant enhancement, approximately four times greater when compared to protein extracted using NaOH.

Proteolysis has a significant impact on protein solubility. Guan et al. (2007) conducted a study to investigate the effect of trypsin on oat protein solubility. In this study, oat protein isolated from oat brans underwent an initial alkali extraction, followed by treatment with trypsin. When the degree of hydrolysis reached 8.3%, the solubility of oat protein after trypsin treatment notably increased to 68.2% at pH 5, in contrast to the 7.3% solubility observed in non-treated protein. Interestingly, higher solubility was achieved when the protein was treated under more alkaline or acidic conditions. Guan et al. (2007) postulated that this enhanced solubility might be attributed to structural modifications, reduction in molecular size, and increased exposure of charged and polar groups to the surrounding aqueous environment.

During the oil extraction process, the solubility of proteins can be affected when they come into contact with solvents. In a study conducted by Yue, Gu et al. (2021), oat protein extraction involved an initial alkali treatment, followed by analysis to determine solubility and other functional properties of the protein. Interestingly, oat protein solubility significantly increased when the oat material was pre-treated with hexane to extract oil. In contrast, research on soy protein, as demonstrated by Sessa et al. (1998), showed a significant increase in the nitrogen solubility index (NSI) with an increase in ethanol concentration. While NSI was approximately 11.0% at 70.0% ethanol, it increased to about 70.0% when the material was treated with absolute ethanol. However, a sharp decrease in NSI was observed at an ethanol concentration of 83.5%. Alcohols have the capacity to induce protein instability by affecting hydrophobic interactions within non-polar residues and disrupting the water structure that interacts with protein molecules. This abrupt change in protein solubility, as reported by Sessa et al. (1998), was explained as a potential phenomenon of protein denaturation.

Heat treatment can significantly impact the functional properties of soluble oat proteins, potentially affecting their suitability for various technical and functional food applications. One consequence of heat treatment is the enlargement of less soluble globulin fractions, characterised by increased hydrophobic amino acid residues. This could explain the reduced emulsifying capabilities observed in emulsions made with heat-treated oats as a base. For instance, a study by Runyon and colleagues (2015) investigated the solubility of oat proteins in response to temperature treatment. Oats subjected to steam treatment at 102 °C for 50 minutes and subsequent drying at 110–120 °C for 50 minutes exhibited a significant reduction in the availability of soluble protein (50 wt. % reduction in soluble proteins), particularly affecting albumins and prolamins more than the globulin fraction. Solubility testing involved the extraction of oat protein from oat flour in a 200 mM sodium phosphate buffer at pH 9.5, containing a protease inhibitor.

1.3.2. Oil and water holding capacity of oat protein / *Auzu proteīnu eļļas un ūdens noturēšanas spēja*

Water-protein interactions predominantly determine the functional properties of the product, comprising water holding capacity, solubility, emulsification, swelling, viscosity, gelation, and syneresis. Water retention substantially impacts the colour, texture, and sensory attributes of the products. Water engages with proteins through various mechanisms, and a substantial quantity of water becomes bound to proteins through hydrogen bonding (Zayas, 1997b).

The binding capacity of water to proteins depends on the amino acid composition. Polar amino acid chains can bind 2 to 3 water molecules, whereas non-polar ones such as alanine or valine can bind a single molecule. Numerous factors, including protein concentration, pH, ionic strength, and denaturation, have been reported to influence water binding capacity. Protein fractions exhibit non-uniform water retention capacities, with albumins and glutelin demonstrating a higher water holding capacity in oats (Zayas, 1997b).

In contrast to the research on water binding, there has been a limited investigation into the mechanism of oil binding. The absorption of oil by proteins is influenced by processing conditions, the protein source, concentration, as well as the type and distribution of the oil. In the case of protein powders, the oil absorption capacity can be affected by particle size, with smaller particles demonstrating increased capacity for oil absorption (Y. Zhang, Sharan, Rinnan, & Orlien, 2021).

Water holding capacity

It was reported that oat protein, obtained by the alkaline extraction method from various fractions of commercially milled oats, retained water in the range of 1.27 to 1.42 g water g^{-1} sample. The protein content in the samples ranged from 75.0% to 97.1%. However, it was observed that the water holding capacity did not correlate with the protein concentration. Samples containing a higher amount of total carbohydrate content demonstrated a significant increase in water holding capacity (Walters et al., 2018). Authors speculated that the water holding capacity is related to the presence of carbohydrates. It is important to highlight that the presented measurements were derived under elevated G-force conditions, specifically at 10 000 × g, which stands in contrast to the conventional practice wherein results are typically obtained under substantially lower G-forces, typically falling within the range of approximately 2 500–3 000 × g.

Mirmoghtadaie et al. (2009) reported comparable findings. In their study, protein concentrates obtained through the alkaline extraction method demonstrated a water holding capacity of 1.27 g water g^{-1} sample. Furthermore, protein modifications, specifically deamidation and succinylation, led to a substantial enhancement in water holding capacity, with values reaching 2.53 and 4.39 g water g^{-1} for deamidated and succinylated protein, respectively.

Conversely, Ma (1983) reported a water holding capacity within the range of 2.70 mL g^{1}. The protein extraction was achieved through the utilisation of the alkaline extraction method.

Enzymatic protein extraction method revealed even higher water holding capacity, reaching up to 3.73 mL g^{-1}, particularly when the protein extraction process involved the pre-treatment of oat brans with amyloglucosidase (Prosekov et al., 2018). Ma and Harwalkar (1984) revealed that oat protein fractions, as fractionated according to the Osborne scheme, exhibit non-uniform water retention capabilities, with albumins and glutelin demonstrating a greater capacity to retain water in oats.

A considerable quantity of water molecules is inherently associated with proteins through the establishment of hydrogen bonds with oxygen and nitrogen atoms, thus constituting the primary hydration shell. These water molecules persist in this arrangement despite undergoing subsequent processing steps. Even after undergoing the lyophilisation process, proteins continue to retain a degree of hydration, typically within the range of 5% to 8% (Murthy, 2021).

The conducted study by Mohamed et al. (2009) reported the amount of “robust” water-binding properties with oat proteins. In this investigation, oat protein extracted from oat flour using the alkaline extraction method was subjected to analysis. This analysis involved the addition of 30% water to the sample weight, followed by an overnight equilibration period and subsequent heating, which yielded results indicating the binding of water within the protein structure.

Oat protein isolated from oat flour by the alkaline extraction method was investigated by adding 30% of water to the sample weight, leaving it overnight to equilibrate and then exposing it to heat treatment. The observed water content retained within the sample was reported as 0.092 g water g^{-1} protein. However, when the protein was subjected to various modifications, including crosslinking, acetylation, acetylated-crosslinking, and succinylation, distinct levels of water binding were observed, with values of 0.045, 0.082, 0.027, and 0.081 g water g^{-1} protein, respectively (A. Mohamed et al., 2009).

Oil holding capacity

Although the binding of water to proteins is primarily attributed to hydrogen bonding forces, it has been proposed that lipids interact with proteins through their binding to the non-polar side chains of proteins (Zayas, 1997a). Consequently, proteins characterised by low solubility and a high degree of hydrophobicity tend to exhibit a relatively high capacity for holding oil. Furthermore, one of the primary mechanisms contributing to the binding capacity of proteins with oil involves the physical entrapment of the oil. An increase in bulk density may also enhance the protein's capacity to bind lipids. Nevertheless, contrasting viewpoints have been reported in some statements. For instance, Kumar et al. (2021) suggested that oat protein may exhibit an enhanced oil binding capacity, primarily attributed to its lower bulk density when compared to other protein isolates, particularly wheat gluten or soy protein isolate. It was hypothesised that the lower bulk density of oat albumins could contribute to their increased fat binding capacity by facilitating the entrapment of oil.

The reported oil binding capacity for oat protein obtained through alkaline extraction fell within the range of 2.25 to 2.80 mL g^{-1} sample (C. Y. Ma, 1983). The oil holding capacity depended on the oat variety used for oat protein extraction, and it was substantially higher than found in wheat gluten or soy protein isolate, which comprised 0.85 mL g^{-1} and 1.83 mL g^{-1} for wheat gluten and soy protein isolate, respectively.

1.3.3. Surface activity and foaming / *Virsmas aktivitāte un putošana*

Another significant functional attribute of proteins is their capacity to create foams, which significantly influence the characteristics, texture, and structure of food products. Foams are encountered in various food applications, either as an integral part of the final product or introduced during the initial stages of production, with the possibility of undergoing further processing before the final product is prepared. They are composed of air bubbles trapped within a continuous liquid phase and require surface-active substances to create and maintain their structure. Foams are classified as thermodynamically unstable systems, and among the surface-active constituents, proteins can play a crucial role in both the formation and preservation of foam structures (Brückner-Gühmann, Heiden-Hecht, Sözer, & Drusch, 2018; Kaukonen et al., 2011).

Numerous factors associated with proteins have been identified as exerting a predominant influence on their surface activity, especially at the molecular level. According to a review by Horbert and Brash (1987) general factors that can be recognised include the protein's size and charge, the protein's structural attributes, and its chemical properties, including amphipathicity, hydrophobicity, and solubility.

These mentioned attributes relate to the amino acid sequences of proteins, which exhibit significant variations among different proteins. The molecular size of a protein is considered to be a factor that potentially results in a greater number of interaction points, particularly at the surface level. It has been postulated that larger molecules possess a higher number of contact points available for interactions upon surface contact. This observation leads to the conclusion that the simultaneous dissociation of all bound points on the surface is unlikely. However, it should be noted that surface activity may not exhibit a straightforward correlation with molecular weight, depending on the source of the raw material. Research conducted on soy protein isolate, for instance, has indicated that smaller molecules exhibit higher solute surface activity, whereas larger, insoluble molecules demonstrate lower activity (Feng, Berton-Carabin, Ataç Mogol, Schroën, & Fogliano, 2021).

Considerable attention has been given to the observation that distinct protein fractions exert varying degrees of influence on the foaming process. An illustrative instance of this phenomenon is evident in the case of oat albumins, which, among the various oat protein fractions, contribute to the highest foaming capacity (Konak et al., 2014). Furthermore, the conducted research on factors influencing oat foaming capacity revealed that lipid-binding

proteins, identified through N-terminal sequencing as tryptophanins, were identified as the principal proteins actively involved in foam formation (Kaukonen et al., 2011). The tryptophanins were predominantly localised within the oat foam phase and were detected within a 15 kDa protein band. Additionally, proteins with higher molecular weights were also discerned within the oat foam, though in lesser quantities compared to those in the 15 kDa range.

In contrast, due to the limited solubility of oat proteins, the formation and stability of films and foams rely on the creation of colloidal aggregates. It has been suggested that characterising the colloidal state of proteins in terms of their size and charge properties is crucial. Ercili-Cura et al. (2015) conducted a study examining the colloidal size and charge of oat protein isolate at pH 7.2 and 9.0, particularly in relation to its surface activity at the air-water interface. Their findings indicated that the dispersions were electrostatically stable, with average particle sizes of approximately 70 nm at pH 7.2 and around 30 nm at pH 9.0. It was hypothesised that oat globulin monomers were adsorbed to the interface and formed various aggregates as multi-layers at pH 7.2, while aggregation was significantly limited at pH 9.0, where the interface was primarily composed of monomers.

In a separate study, oat protein isolate obtained through alkaline extraction, with prior lipid removal using SC-CO_2, exhibited the ability to foam rapidly and disperse gas bubbles effectively. This foaming capacity was attributed to the formation of a thick steric protein layer, serving as a stabilising mechanism (Brückner-Gühmann et al., 2018). However, this phenomenon was primarily observed at pH 7. Lowering the pH to 4 required additional oat protein modification, particularly through enzymatic hydrolysis, to maintain foam properties at a comparable level. Interestingly, the study noted that when modified using alcalase, both foaming capacity and foam stability were lower compared to modifications achieved through tryptic hydrolysis.

It is important to note that lipids, which often accompany protein during the extraction process, tend to disrupt foaming properties. However, Kaukonen et al. (2011) found that lipid composition significantly influences both the foam capacity and stability in oat protein extracts. Oat extracts were obtained by extracting water-soluble components from oats, primarily consisting of soluble oat protein. Their study concluded that non-polar lipids negatively impact foam quality, whereas the presence of polar lipids has a comparatively minor impact.

Furthermore, oat extracts defatted with CO_2 exhibited significantly higher foaming capacity (averaging 137%) compared to those defatted with hexane (averaging 35%). It is worth considering the influence of other components typically found alongside oat protein after extraction, such as β-glucans, which can directly impact foam characteristics by altering the solution's viscosity (Kaukonen et al., 2011).

It can be concluded that oat protein's foaming properties are generally comparable to those of other plant-based proteins. For instance, oat protein isolate obtained through alkaline extraction and prior defatting displayed a foaming capacity ranging from 85% to 120%. In contrast, wheat gluten and soy protein isolate exhibited foaming capacities at a range of 100% and 135%, respectively (C. Y. Ma, 1983). As expected, protein concentrates from non-defatted oat groats had significantly lower foaming capacity, measuring at approximately 25%.

1.3.4. Factors affecting the functionality of oat proteins / *Faktori, kas ietekmē auzu proteīnu funkcionalitāti*

In a study conducted by Ma et al. (2003), the impact of temperature on oat protein aggregation and gelation was investigated. Oat globulins, extracted using 1.00 *M* NaCl, were subjected to thermal treatment in a 0.01 *M* phosphate buffer (pH 7.4) containing 1.00 *M* NaCl, and heated at 110 °C for 30 minutes. Subsequently, the proteins were separated by centrifugation, dialysed against distilled water, and freeze-dried.

The results indicated that significant protein denaturation occurred in the insoluble protein fraction (underflow in centrifugation), while relatively little denaturation was observed

in the soluble fraction (overflow in centrifugation). This denaturation was characterised by a reduction in the α-helical structure and an increase in β-sheets in both phases of centrifugation. Furthermore, the study found that the Raman spectral characteristics of oat globulins showed minimal changes following thermal treatment, suggesting that oat globulins exhibit high thermal stability. Oat protein displays remarkable resistance to temperature-induced denaturation. In a study by Marcone, Kakuda, and Yada (1998), the denaturation temperature of oat globulin was investigated. The researchers focused on examining thermal stability, which depended on various structural factors such as amino acid composition, compact packing of proteins, protein-protein interactions, intramolecular linkages, and interactions. Notably, oat globulin exhibited a thermal transition temperature of 112 °C, which was the highest among all the proteins studied.

The size of a protein plays a crucial role in determining its solubility characteristics, where larger protein molecules generally tend to be less soluble (Feng et al., 2021). This phenomenon is linked to the analysis of free amino groups within proteins, where insoluble fractions with higher molecular masses typically have fewer free amino groups compared to monomeric fractions found in soluble fractions. Furthermore, the size of a protein molecule and its predisposition to form aggregates are strongly influenced by the protein's concentration.

This concept is applicable to oat protein, which has a propensity to form aggregates under specific conditions. For instance, oat protein isolated through a process involving NaOH treatment followed by isoelectric precipitation exhibited a tendency to form aggregates and increase in size when the protein concentration reached 1 mg mL^{-1} in aqueous media (G. Liu et al., 2009). Notably, self-assembly became notably more pronounced at this concentration, whereas at a concentration of 0.5 mg mL^{-1}, there was only a slight increase in protein size compared to the initial protein molecule size. This suggests that higher concentrations of oat protein in aqueous solutions can alter the protein's stability, leading to the formation of protein aggregates through the association of neighbouring proteins (G. Liu et al., 2009), particularly as the ionic strength of the solution increases (Durand et al., 2002). It is important to highlight that a concentration of 1 mg mL^{-1} is relevant to industrial applications that incorporate oat protein as an ingredient, and it should be thoroughly considered and evaluated.

1.4. Oat applications utilising protein / *Auzu lietojumi, izmantojot proteīnu*

1.4.1. Commercial oat protein / *Komerciāls auzu proteīns*

Despite its well-studied functional properties and relatively high nutritional value, concentrated oat protein is not widely available in the market. Some attempts to commercialise such protein have been made, primarily in Scandinavian countries. Currently, the product by Lantmännen, known as "PrOatein"[1] is the only oat protein concentrate available in the market. This product contains over 50% protein and is also rich in oil and maltodextrins, with approximately 16–19% and 20–24%, respectively. The company employs a patented technology to extract oat protein concentrate from oat brans, involving a wet milling process combined with alpha-amylase treatment to remove insoluble fibre. During this process, other oat derivatives such as oat beta-glucan or oat dextrin may also be obtained. The product finds applications in various industries, including bakery, beverages, and meat substitutes. Notably, oat protein properties differ significantly from traditional plant-based proteins like soy or pea, particularly in terms of oil content, structure, and functional properties.

There were also attempts to establish production units in the USA, such as Oat Tech, Inc., which produced similar oat protein products along with some streams of oat dextrin. For instance, their product "Oat Protein 55" produced using patented technology, had a

[1] Lantmännen Oats. ("PrOatein Oat Protein a Plant-Based Protein Extract," n.d.), accessed 2023-10-01, URL: https://www.lantmannenoats.com/proatein/.

concentration of about 55% (Whalen, 2013). However, these attempts were not sustainable, and limited information is available to discuss the issues that led to the termination of Oat Tech's activities.

Recent research in dry fractionation techniques has shown promise in achieving higher protein purity, with concentrations of up to 73% (Sibakov et al., 2011). Fazer, a company in Finland, has obtained a licence to use a technology from the VTT research centre[2], although such highly concentrated oat protein is not yet commercially available.

The limited instances of industrial-level oat protein extraction suggest that current methods may not be suitable for large-scale production. Moreover, the functional limitations of oat protein quality may also constrain its applicability in various products. However, ongoing efforts to enhance protein functionality through modifications indicate a commitment to finding the right path for successful commercialisation. The next chapter will review current advancements in oat protein modification methods and explore potential applications.

1.4.2. Solid products / *Cietie produkti*

Oat protein, primarily intended for the food industry, presents considerable potential for utilisation in diverse industries. Its unique characteristics offer advantages to industries seeking plant-based protein properties, extending its applicability beyond traditional food uses. Oat protein's monomeric structure and relatively high protein content in groats distinguish it from other proteins like soy or pea, making it a valuable choice for industries requiring a monomeric protein structure, as for example in biomaterials.

For instance, amyloid structures could successfully be formed from purified oat globulins (J. Zhou et al., 2022). Oat globulins were extracted by passing defatted oat flour through alkaline extraction at pH 10. Further solution was adjusted to pH 7, and protein was separated, passed through 5% NaCl treatment, readjusted to pH 4.5, dialysed against water using 3.5 kDa membranes and freeze-dried. The obtained globulin fraction was subjected to acidic treatment at pH 2 and finally freeze-dried. Such a procedure gave an irreversible amyloid structure, which was used to produce state-of-the-art membranes for water purification, heavy metal removal (those outperformed the most frequently used milk-based β-lactoglobulin amyloid fibrils). Applications in nanotechnology, such as coated Au-nanoparticle interdigital electrodes were also reported, which might be applicable in soft robotics or biodegradable electronics (Y. Li et al., 2020; Z. Ma et al., 2021) or being pressure sensitive to be used in pressure-electric sensors (J. Zhou et al., 2022).

One promising trend in the food industry involves the use of oat protein concentrates and isolates, particularly in conjunction with extrusion technology. This trend is driven by the increasing demand for plant-based protein sources due to various factors, including concerns about human health and the demand for more sustainable food options (Pietsch, Bühler, Karbstein, & Emin, 2019).

One of the emerging trends in extrusion technology is high-moisture extrusion, where the moisture content of the input material to the extruder exceeds 40%. This approach offers several benefits, including reduced energy consumption and improved quality of the resulting texturized products (J. Zhang et al., 2019). It can produce texturized products with a rich, fibrous structure and a springiness similar to real animal meat (J. Zhang, Liu, Jiang, Faisal, & Wang, 2020).

Limited protein solubility is considered an advantageous characteristic when aiming to create the desired fibrous structure through extrusion (Geerts, Dekkers, Van Der Padt, & Van

[2] News Powered by Cision. ("Fazer Turns over a New Leaf in the Story of Finnish Oats - A Cutting-Edge Innovation to Boost Oat Exports," 2015), 10 June 2015, accessed 2023-10-22, URL: https://news.cision.com/fazer-group/r/fazer-turns-over-a-new-leaf-in-the-story-of-finnish-oats---a-cutting-edge-innovation-to-boost-oat-ex,c9789706.

Der Goot, 2018). Rather, the ability to retain water is crucial in achieving this structure during the extrusion process. Given these properties inherent to oat protein, high-moisture extrusion appears to unlock significant potential for oat protein in this context. For instance, air-separated oat protein concentrate (39.5% protein, 33.0% starch on a wet basis) was subjected to high moisture extrusion with prior protein modification (Pori et al., 2022). The protein samples before extrusion were treated with transglutaminase and a combination of transglutaminase and protein-glutaminase and freeze-dried. The native, control samples after extrusion revealed a doughy texture which is generally undesirable in plant-based meat structures. Enzymatic pre-treatment improved structure to some extent. The extrudate produced of protein with the highest solubility, in particular, treated with a combination of transglutaminase and protein-glutaminase, had no fibrous structure. Preheating of the samples before the enzyme treatment at 95 °C for 15 minutes has improved the structure of all extrudates. It is worth mentioning that the samples comprised a substantial amount of starch, subsequently altering the structure of the extrudate.

Oat protein could successfully be used as a supplementary ingredient for extrusion. For instance, the extrusion of pea-oat blend to produce fibrous meat analogues was reported (Kaleda et al., 2020). A similar attempt was demonstrated in high moisture extrusion wherein a blend of pea and de-starched oat protein concentrate was used in the composition (Immonen, Chandrakusuma, Sibakov, Poikelispää, & Sontag-Strohm, 2021). For this purpose, oat protein was produced by treating oat protein concentrate with α-amylase, with subsequent centrifugation and drying of the resulting pallet. Such a method increased protein concentration in oat from an initial 25.6% to 33.1% and resulted in a fibrous structure of the extruded blend. Oat protein's potential use in burgers has also been a subject of investigation (Ball, Wyatt, Coursen, Lambert, & Sawyer, 2021).

1.4.3. Liquid and semi-liquid products / *Šķidrie un pussķidrie produkti*

Oat protein concentrates have the potential to serve as an additional source of oat protein in dairy alternatives. However, commercially available oat protein concentrates currently exhibit poor techno-functionality in liquid and semi-solid applications (Spaen & Silva, 2021). Several attempts have been made to incorporate oat protein concentrate into yoghurt production. Brükner et al. (2019), for instance, reported achieving the gelling state by enriching yoghurt with oat protein obtained through alkaline extraction. Protein was introduced and fermented by inoculating the yoghurt culture (*Lactobacillus delbrückii* subspecies *Bulgaricus* and *Streptococcus thermophilus)*. The functional properties of this particular oat protein, such as gel strength or protein solubility were reported as poor, with little compatibility with skimmed milk.

In contrast, oat protein concentrate obtained through air separation was noted as a suitable replacement for skim milk powder in yoghurt production. A subsequent study explored yoghurt made using oat protein concentrate obtained through air separation, which was characterised by a protein content of 28.3% and starch content of 45.3% (Brückner-Gühmann, Banovic, & Drusch, 2019). The resulting samples were described as soft fluid gels. Although the oat protein concentrate exhibited potential as a functional ingredient in lactic acid-fermented oat gels, it was ultimately concluded that the presence of starch in the oat protein concentrate significantly influenced the rheological properties of the gels.

Oat protein was investigated as a gel-forming material. Protein isolated through alkaline extraction passed acidification induced by using glucono-δ-lactone at concentrations from 3 to 15%. (C. Yang, Wang, & Chen, 2017). Obtained active monomers of oat protein produced polymer-like percolating structures as small blocks through abundant cross-linking points. The concentration of glucono-δ-lactone at a range of 10% resulted in a gel with a compact network structure with small pores, and comparable to the strength of egg white. The gel was

successfully used to encapsulate enzymes and probiotics, as it resisted acidic juice and pepsin digestion.

Research suggests that oat protein's techno-functionality can be improved through enzymatic or chemical treatments. These approaches should be considered when developing oat protein ingredients for commercial use and oat protein-enriched dairy alternatives. Detailed methods for enhancing oat protein functionality are discussed in subsequent chapters.

1.4.4. Products obtained through chemical alteration / *Produkti, kas iegūti ķīmiskas pārveidošanas rezultātā*

Chemical protein modification can be considered a method that enables the improvement of the properties of raw materials. The strengths of the chemical process can be seen in its high efficiency and ease of control (Z. Wang, Zhang, Zhang, Ju, & He, 2018). In some cases, it may even surpass mechanical and enzymatic processes in terms of industrial applicability when dealing with large-scale process implementation (Zhao et al., 2017).

Modification by anhydrides through acetylation and succinylation

The presence of anhydrous organic acids initiating protein conformational and functional changes has been investigated by Zhao et al. (2017). Treating oat protein isolate with acetic and succinic anhydrides at a ratio ranging from 0.2 to 1.0 (g g^{-1}) to protein, followed by pH stabilisation at 9, and subsequent neutralisation at pH 7, resulted in acetylated and succinylated oat protein isolate. As the ratio of anhydrides increased, the hydrophobicity of oat protein decreased, irrespective of the type of anhydride, although the decrease was more pronounced with succinylation. However, when the anhydride-protein ratio was increased to 0.8, the hydrophobicity increased from approximately 40% to about 80% for acetylation, whereas succinylation continued to exhibit a decrease in hydrophobicity. The authors speculated that such a significant increase was possible due to the production of positive charges, which replaced the neutral charges during the dissociation of subunits.

Another investigation comes from Mirmoghtadaie et al. (2009), where the authors claimed an increase in the negative charge of the net after succinylation was applied to oat protein isolate. The amount of anhydride used for the trials was set at 20 to 100 g g^{-1} of oat protein isolate.

It should be noted that hydrophobicity depends on pH, as demonstrated with succinylated canola protein, indicating that hydrophobicity is influenced by both pH and, to some extent, the presence of salts (Paulson & Tung, 1987). While the acylation of oat protein has not improved the molecular weight, succinylation revealed marked changes in molecular mass by increasing it gradually (Zhao et al., 2017). Acetylation and succinylation induced conformational changes in protein, although a stronger influence was assigned to the succinylation process, due to stronger electrostatic repulsion interactions in combination with the steric hindrance. The modification of oat protein led to a decrease in β-sheets, while α-helix and random coil structures increased.

These aforementioned protein conformations significantly improved the functional properties oat protein. For instance, the measured nitrogen solubility of succinylated oat protein increased from 22.9 to 86.8%, water holding capacity from 1.27 to 4.39 g g^{-1}, and oil holding capacity from 1.73 to 2.24 g g^{-1} compared to native protein. On the other hand, foaming capacity decreased. It was speculated that the decrease in foaming properties was caused by the excessive increase in negative charge, which subsequently reduced protein-protein interactions

and prevented the formation of elastic film at the air-liquid interface (Mirmoghtadaie et al., 2009).

Deamidation

Deamidation might be considered as an alternative way to improve the functional properties of protein. Removing amide groups from asparagine and glutamine residues increases negatively charged carboxyl groups, and consequently, the isoelectric point decreases, simultaneously enhancing solubility and other functional properties of proteins due to more acidic conditions, yet still mildly (Hamada & Marshall, 1989). There are two common methods for initiating protein deamidation, which will be briefly discussed below: chemical and enzymatic approaches.

Chemical deamidation encompasses various methods, including acid deamidation, alkali deamidation, and salt deamidation, which can modify proteins in several ways (C. Cui et al., 2013). These modifications involve increasing electrostatic repulsion, stretching protein structures, altering charge density, and disrupting hydrogen bonds. Such treatments result in the unfolding of protein conformations and the strengthening of interactions between proteins and water. In previous investigations, hydrochloric acid has been widely employed for protein deamidation.

The mild acidic protein deamidation process results in a reduction in the size of oat globulins. In this process, 0.50 *N* HCl was used at a ratio of 1 to 20 to hydrolyse the oat protein for two hours at 70 °C. The reaction was then cooled to discontinue it, followed by precipitation and centrifugation, and subsequent neutralisation. The obtained sample was subjected to observation using SDS-gel electrophoresis, revealing that the oat protein bands at around 60 kDa (native protein isolate) disappeared, while bands at 40 kDa and 20 kDa were significantly weakened. This led to the conclusion that acid hydrolysis reduced the size of oat globulin, resulting in the production of smaller-sized peptides (Mirmoghtadaie et al., 2009).

Enzymatic deamidation was considered a preferable protein modification process compared to acidic deamidation, as it allows for selectivity and milder conditions, such as a neutral pH and lower temperatures (Hamada & Marshall, 1989; Suppavorasatit, De Mejia, & Cadwallader, 2011). The deamidation of oat protein was performed by Jiang et al. (2015), using enzymatic treatment, specifically with food-grade protein glutaminase. The reaction was conducted under neutral pH and low salt conditions, resulting in a degree of deamidation of up to 59%. The degree of deamidation was determined by measuring the concentration of ammonia. However, Jin et al. (2022) recommended mass spectrometric analysis, which provides a more comprehensive characterisation of deamidation sites. The degree of deamidation had an impact on the solubilisation level, with higher deamidation leading to greater solubilisation. This effect was observed to reach up to 95% solubilisation at a temperature of 50 °C when the degree of deamidation reached 59%. Interestingly, the increase in temperature from 21 °C to 50 °C had a noticeable effect when the degree of deamidation was at 42%. However, when it reached the maximum level tested in the trial, 59%, the temperature had little impact on the degree of deamidation.

Furthermore, the effect of deamidation significantly enhanced the emulsifying properties of oats. Examining the emulsion structure under a microscope revealed a negative correlation between the degree of deamidation and the size of oil droplets. A higher degree of deamidation resulted in smaller oil droplets in the solution, and at the highest examined degree of deamidation, a uniform distribution of oil droplets was observed (Jiang et al., 2015). This resulted in a stable emulsion that remained unchanged for up to 30 days, preventing the formation of layers typically observed at lower degrees of deamidation. The authors hypothesised that the achieved emulsion stability was due to protein solubilisation, which effectively coated the oil droplets.

The results of oat deamidation are similar and comparable to those of extensively researched soy protein deamidation, where the enzymatic improvement of solubility and

emulsifying properties was observed (Suppavorasatit et al., 2011). The degree of deamidation could be increased by combining chemical treatment with subsequent enzymatic proteolysis (Hamada, 1992) or by using enzyme blends such as transglutaminase and protein-glutaminase, as recently reported for oat protein by Pori et al. (2022).

Increase in protein functionality through interaction with carbohydrates

The interaction between proteins and carbohydrates is crucial, particularly considering the fact that carbohydrates are the predominant component in crops. Glycation is a widely used method for modifying proteins, making it popular due to its applicability to a broad range of proteins (Wu, Liu, & Hu, 2022). When proteins are dry-heated, they undergo intensive interaction with carbohydrates, resulting in a significant reduction in free amino acids (Feng et al., 2021). Carbohydrate sources for glycation vary, with dextran and maltodextrin being common choices (Akhtar & Ding, 2017; Nakamura, Kato, & Kobayashi, 1991; Wong, Day, McNaughton, & Augustin, 2009). Shorter chain carbohydrates have a more noticeable impact on reducing free amino acids in the raw material, which serves as an indicator of protein glycation levels. Limited recent research has explored the interactions between oat protein and carbohydrates.

In the study of Zhong et al. (2019), it was found that oat protein showed limited reactivity with saccharides. However, denatured oat protein, characterised by exposed lysine residues in larger quantities, exhibited increased reactivity when in proximity to β-glucan polysaccharide derived from *Pleurotus ostreatus*. Moreover, increasing the polysaccharide to a protein ratio beyond 1:3 did not further increase reactivity, which was attributed to steric hindrance from the polysaccharides limiting the reaction. This protein conjugation process was noted to potentially alter the functional properties of the protein. Furthermore, oat protein's solubility significantly increased after covalent binding, due to two conformational changes. The presence of β-glucan polysaccharide increased the hydrophilic groups, enhancing steric stabilisation, and shifted the pH to a more acidic region, resulting in a wider solubility range (Zhong et al., 2019). Glycation also supported the emulsification of oat protein isolate that was conjugated with dextran. The glycation reaction at 90 °C and pH 9 significantly improved the emulsifying properties of the oat isolate, as well as its stability when subjected to changes in pH and salt levels (Bei Zhang, Guo, Zhu, Peng, & Zhou, 2015).

Protein solubility improvements with the presence of dextran were observed in various crops, such as rice, as reported by Cheng et al. (2018). In the case of rice protein, its solubility increased significantly, up to 7.5 times when compared to the original source, and its emulsifying and foaming properties also showed enhancements, with factors increasing from 1.7 to 2.2. Notably, the increased ratio of dextran to protein was strongly correlated with solubility. However, it is important to note that protein solubility started to decrease when the temperature was raised from 95 °C to 100 °C and beyond, a phenomenon attributed to protein refolding and precipitation primarily driven by protein hydrophobic interactions.

Although glycation is a relatively effective process for enhancing the functional properties of proteins, it can have drawbacks such as undesired changes in colour and the formation of potentially toxic components such as furfural, hydroxymethylfurfural, and furosine, which can limit protein applicability (Guerra-Hernandez, Leon Gomez, Garcia-Villanova, Corzo Sanchez, & Romera Gomez, 2002; Wu et al., 2022). Additionally, the involvement of amino acids like lysine in these interactions raises concerns about a potential decrease in the protein's nutritional value. Another concern is the high temperatures used in the glycation process, which can expose vital food components like vitamins to prolonged treatment.

Summary of literature review / *Literatūras apskata kopsavilkums*

Oats have great potential as a source of plant protein concentrate and isolate due to their excellent amino acid composition and higher protein content compared to other cereals. Protein extraction from oats involves separating proteins from other kernel compounds, and two main methods are dry milling with air separation and wet extraction, which is typically solvent-based. Given the high lipid content in oats, it is essential to consider lipid extraction as a crucial step during oat processing.

Oat protein's functional properties are generally modest, with solubility being pH-dependent, and it varies based on the extraction method. However, it performs comparably with other plant-based proteins in terms of liquid holding capacity and foaming ability.

Despite its beneficial nutritional value, concentrated oat protein is not widely available, and current extraction methods are insufficient for large-scale production. This limits protein applicability in various products.

The food industry is showing a rising interest in oat protein concentrates and isolates due to the increasing demand for plant-based protein sources. A promising trend involves using oat protein concentrates and isolates, often combined with extrusion technology. However, current extraction methods, which rely on alkaline extraction and precipitation, have limitations that impact protein properties and nutritional value.

There is a need for the development of new extraction and concentration methods for oat protein to support sustainable and safe production processes and meet the rising demand for plant-based protein products. Consequently, one potentially effective approach for oat protein production could involve using enzyme-assisted extraction and purification, which has the potential to be implemented on an industrial scale.

The **aim** of the doctoral thesis was to develop enzyme-assisted aqueous extraction methods to obtain oat protein concentrates, evaluate the functional properties of the obtained protein concentrates, and determine their suitability for further processing, including but not limited to wet extrusion.

Tasks of the present research are as follows:

- to identify methods suitable for oat protein enzyme-assisted aqueous extraction from commercial whole oat flakes and fine oat flour;
- to identify suitable defatting methods for oat protein concentrates obtained from commercial oats and oat flour;
- to evaluate the redistribution of amino acids in obtained oat protein concentrates and side products, in particular, fibre;
- to evaluate characteristics of obtained protein concentrates and investigate functional properties of obtained oat protein concentrates;
- to determine the extrusion parameters for oat protein concentrate;
- to investigate the structure and colour of the obtained oat extrudate.

Auzām ir liels potenciāls kā augu izcelsmes proteīna koncentrāta un izolāta avotam, jo tām ir izcils aminoskābju sastāvs un augstāks olbaltumvielu saturs salīdzinājumā ar citiem graudaugiem. Proteīna ekstrakcija no auzām ir proteīna atdalīšana no citiem grauda kodola komponentiem, un divas galvenās metodes ir sausā malšana ar aeroseparāciju un mitrā ekstrakcija, kas parasti tiek veikta ar šķīdinātāju. Ņemot vērā augsto lipīdu saturu auzās, ir svarīgi uzskatīt lipīdu ekstrakciju par būtisku auzu apstrādes posmu.

Auzu proteīna funkcionālās īpašības parasti ir pieticīgas, šķīdība ir atkarīga no pH un var mainīties atkarībā no ekstrakcijas metodes. Tomēr šķidruma noturēšanas un putošanas spējas ziņā tas ir salīdzināms ar citām augu izcelsmes olbaltumvielām.

Neraugoties uz tā labvēlīgo uzturvērtību, koncentrēts auzu proteīns nav plaši pieejams, un pašreizējās ekstrakcijas metodes nav piemērotas liela apjoma ražošanai. Tas ierobežo proteīna pielietojamību dažādos produktos.

Pārtikas rūpniecībā pieaug interese par auzu proteīna koncentrātiem un izolātiem, jo pieaug pieprasījums pēc augu izcelsmes olbaltumvielu avotiem. Daudzsološa tendence ir izmantot auzu proteīna koncentrātus un izolātus, bieži vien kombinējot ar ekstrūzijas tehnoloģiju. Tomēr pašreizējām ekstrakcijas metodēm, kas balstās uz sārmu ekstrakciju un izgulsnēšanu, ir ierobežojumi, kas ietekmē proteīna īpašības un uzturvērtību.

Ir nepieciešams izstrādāt jaunas auzu proteīna ekstrakcijas un koncentrēšanas metodes, lai atbalstītu ilgtspējīgus un drošus ražošanas procesus un apmierinātu augošo pieprasījumu pēc augu izcelsmes olbaltumvielu produktiem. Tādējādi viena no potenciāli efektīvām auzu proteīna iegūšanas metodēm varētu būt fermentatīvā ekstrakcija un attīrīšana, ko varētu īstenot rūpnieciskā mērogā.

*Promocijas darba **mērķis** bija izstrādāt fermentatīvās ūdens ekstrakcijas metodes auzu proteīna koncentrāta iegūšanai, novērtēt iegūto auzu proteīna koncentrātu funkcionālās īpašības un noteikt to piemērotību turpmākai pārstrādei, tostarp, mitrai ekstrūzijai.*

*Šim pētījumam ir šādi **uzdevumi**:*

- *identificēt metodes, kas ir piemērotas auzu proteīna fermentatīvai ūdens ekstrakcijai no rūpnieciskām pilngraudu auzu pārslām un smalkā maluma auzu miltiem;*
- *identificēt piemērotas attaukošanas metodes no rūpnieciskām auzām un auzu miltiem iegūtiem auzu proteīna koncentrātiem;*
- *novērtēt aminoskābju pārdalījumu iegūtajos auzu proteīna koncentrātos un blakusproduktos, jo īpaši šķiedrvielās;*
- *novērtēt iegūto proteīna koncentrātu raksturīpašības un izpētīt iegūto auzu proteīna koncentrātu funkcionālās īpašības;*
- *noteikt auzu proteīna koncentrāta ekstrūzijas parametrus;*
- *izpētīt iegūtā auzu ekstrudāta struktūru un krāsu.*

2. MATERIALS AND METHODS / *MATERIĀLI UN METODES*

2.1. Time and place of research / *Pētījumu laiks un vieta*

The present research was performed between 2019 and 2024. The place and type of the experiments conducted were:

- Latvia University of Life Sciences and Technologies (LBTU), Faculty of Agriculture and Food Technology, Food Institute – protein extrusion, characterisation of raw materials and obtained protein quality parameters.
- Ltd JP Biotechnology – protein extraction.
- LBTU DPP Institute of Horticulture – protein defatting by supercritical CO_2.
- Institute of Agricultural Resources and Economics (AREI) – characterisation of raw materials and obtained protein quality parameters.
- Latvian State Forest Research Institute "Silava" – molecular weight determination.

2.2. Description of materials / *Materiālu apraksts*

The **object** of the research is: whole grain oat flakes, fine oat flour with reduced fibre content (oat flour), commercial enzymes (α-amylase (from *Bacillus Licheniformis)*, complex enzymes (from *Trichoderma reesei)* with main xylanase and side β-glucanase activities), extrudate of the oat protein concentrate. A description of the materials is presented in tables 2.1–2.3 below.

Table 2.1. / *2.1. tabula*

Description of oat flakes and oat flour used in the study as an initial raw material /
Pētījumā kā sākotnējās izejvielas izmantoto auzu pārslu un auzu miltu raksturojums

Raw material / *Izejviela*	**Chemical composition, g 100 g^{-1} in DM /** *Ķīmiskais sastāvs, g 100 g^{-1} sausnā*	**Physical specifications /** *Fizikālās īpašības*	**Producer /** *Ražotājs*
Whole oat flakes / *Pilngraudu auzu pārslas*	• Crude protein / *Kopproteīns* 17.6 g, • Fats / *Koptauki* 5.7 g, • Crude fibre / *Kopējās šķiedrvielas* 2.13 g, • Carbohydrates / *Ogļhidrāti* 59.2 g, • β-glucans / *β-glikāni* 4.8 g, • Salt / *Sāls* 0.01 g	• Heat treated / *Termiski apstrādāts* • Moisture content / *Mitruma saturs* 9.5%	Dobeles dzirnavnieks, SIA (Latvia / *Latvija*)
Fine oat flour / *Smalkā maluma auzu milti*	• Crude protein / *Kopproteīns* 10.4 g; • Carbohydrates / *Ogļhidrāti* 78.1 g, of which sugar / *no kuriem cukuri* 0.4 g; • Fats / *Koptauki* 6.21 g, of which saturated fatty acids / *no kuriem piesātinātās taukskābes* 1.2 g; • Dietary fibre / *Šķiedrvielas* 2.7 g, of which β-glucan / *no kurām β-glikāni* 1.56 g	• Moisture content / *Mitruma saturs* 11.6%, • Particle size distribution / *Daļiņu izmēra sadalījums:* 1000 μm - 0–1 % 300-1000 μm - 0–5 % < 300 μm - 94–100 % • Heat treated / *Termiski apstrādāts*	Helsinki mills (Vaasa, Finland / *Vāsa, Somija*)

Table 2.2. / *2.2. tabula*

Specification of commercial enzymes used in the study /
Pētījumā izmantoto komerciālo enzīmu raksturojums

Parameters / *Rādītāji*	**Enzyme trade mark /** *Enzīmu preču zīme*	
	HSAL	Grainzyme FL
Main activities / *Galvenās aktivitātes*	α-amylase / *α-amilāze* – 40 000 u mL^{-1}.	Xylanase / *ksilanāze* – 12 000 u mL^{-1}; β-glucanase / *β-glikanāze* – 5 000 u mL^{-1}; Cellulase / *celulāze* – 1 000 u mL^{-1}.
Optimal working temperature / *Optimāla darba temperatūra*	94–98 °C	58–66 °C
Optimal working pH / *Optimāls darba pH*	5.8–7.0	5.5–6.5
Strain / *Celms*	*Bacillus Licheniformis*	*Trichoderma reesei*
Producer / *Ražotājs*	Suntaq International, China.	Suntaq International, China.

Table 2.3. / *2.3. tabula*

List of materials and kits used in the study /
Pētījumā izmantoto materiālu un komplektu saraksts

Materials, chemicals, kits / *Materiāli, ķīmiskās vielas, komplekti*	**Producer /** *Ražotājs*	**Country /** *Valsts*
• NaCl (Sodium chloride / *Nātrija hlorīds*), NaCl ≥ 97%, Na ≤ 0.39%, H_20 ≤ 0.25%, $K_4[Fe(CN)_6]\cdot 3H_2O$ ≤ 10 mg kg^{-1}	Artemsol	Ukraine / *Ukraina*
• NaOH (Sodium hydroxide / *Nātrija hidroksīds*)	Sigma	Germany / *Vācija*
• HCl (Hydrochloric acid / *Sālsskābe*)	Sigma	Germany / *Vācija*
• Ethanol / *Spirts* 96.4 % (v/v)	Kalsnava elevators	Latvia / *Latvija*
• $CO(NH_2)_2$ (Carbamide / *Karbamīds*)	Enola	Latvia / *Latvija*
• $CH_3(CH_2)_{11}OSO_3Na$ (Sodium dodecyl sulphate / *Nātrija dodecilsulfāts*)	Enola	Latvia / *Latvija*
• $C_4H_{10}O_2S_2$ (DL-Dithiothreitol / *DL-ditiotreitols*)	Sigma	Germany / *Vācija*
• Protein 230 Gel Matrix / *Protein 230 gēla matrica*: Trometamol / *Trometamols*, 0.50–1.50, % (w/w), CAS Number / *CAS numurs* 77-86-1	Agilent Technologies Manufacturing GmbH & Co. KG	Germany / *Vācija*
• Protein 230 Dye Concentrate / *Protein 230 krāsvielu koncentrāts*: Dimethyl sulfoxide / *Dimetilsulfoksīds*, 80–100 % (w/w), CAS Number / *CAS numurs* 67-68-5 Sodium dodecyl sulphate / *Nātrija dodecilsulfāts* 3–7 % (w/w), CAS Number / *CAS numurs* 151-21-3	Agilent Technologies Manufacturing GmbH & Co. KG	Germany / *Vācija*
• Protein 230 ladder / *Protein 230 marķieris*: Glycerol / *Glicerīns* 7–137 % (w/w), CAS Number / *CAS numurs* 56-81-5, Trometamol / *Trometamols* 0.50–1.57 % (w/w), CAS Number / *CAS numurs* 77-86-1 Lithium dodecyl sulphate / *Litija dodecilsulfāts* 0.1–17.0 % (w/w), CAS Number / *CAS numurs* 2044-56-6	Agilent Technologies Manufacturing GmbH & Co. KG	Germany / *Vācija*

Continuation of Table 2.3. / 2.3. *tabulas turpinājums*

Materials, chemicals, kits / *Materiāli, ķīmiskās vielas, komplekti*	**Producer** / *Ražotājs*	**Country** / *Valsts*
• Protein 230 Sample Buffer / *Protein 230 parauga buferis*: Lithium dodecyl sulphate / *Litija dodecilsulfāts* 1–5 % (w/w), CAS Number / *CAS numurs* 2044-56-6, Trometamol / *Trometamols* 1–5 % (w/w), CAS Number / *CAS numurs* 77-86-1	Agilent Technologies Manufacturing GmbH & Co. KG	Germany / *Vācija*
• Soy protein concentrate crude protein / *Sojas proteīna koncentrāts kopproteīns* 70.57% (dry basis / *sausnā* N × 6.25), moisture / *mitrums* 6.74%, ash / *pelni* 5.74 % (dry basis / *sausnā*), fat / *tauki* 0.70 %, particle size (100 mesh)/ *daļiņu lielums (100 sieta acs)* 95%.	Shandong Yuxin bio-tech co., ltd	China / *Ķīna*

2.3. Methods for the chemical characterisation of materials / *Metodes materiālu ķīmiskajai raksturošanai*

The following methods were applied to characterise the samples: crude protein LVS EN ISO 20483:2014, moisture content ISO 6496:1999, crude fibre ISO 5498:1981, fats ISO 6492:1999, amino acids LVS EN ISO 13910-2005.

2.4. Structure of the research / *Pētījuma struktūra*

The research comprises three general stages described in Table 2.4 below.

Table 2.4. / *2.4. tabula*

Description of the research stages / *Pētījuma posmu apraksts*

Stage / *Posms*	**Description** / *Apraksts*
Stage I / *I posms*	Enzymatic protein extraction from oat flakes and oat flour / *Enzimātiskā proteīnu ekstrakcija no auzu pārslām un auzu miltiem* Influence of 0.1 *M* NaCl solution on protein yield / *0,1 M NaCl šķīduma ietekme uz proteīnu iznākumu* Study of oat protein defatting / *Auzu proteīnu attaukošanas izpēte*
Stage II / *II posms*	Research of the characteristics and functional properties of oat protein concentrates / *Auzu proteīna koncentrātu īpašību un funkcionālo īpašību izpēte*
Stage III / *III posms*	Extrusion of oat protein concentrate and evaluation of the extrudate functional properties / *Auzu proteīna koncentrāta ekstrūzija un ekstrudāta funkcionālo īpašību novērtēšana*

2.5. Stage I of the research / *Pētījuma I posms*

In Stage I, the oat protein concentrates were obtained from oat flakes and oat flour enzymatically hydrolysing starch and non-starch polysaccharides by subsequent protein concentration through centrifugation. In addition, the research aimed to explore the effects of changes in ionic conditions on both protein yield and characteristics. The subsequent studies were subjected to a detailed evaluation.

Utilising whole oat flakes as a raw material:

- enzymatic hydrolysis of starch and non-starch polysaccharides with subsequent centrifugation steps to reduce the suspended fibre and separate the protein;
- evaluation of the ionic change influence on protein yield.

Utilising fine oat flour as a raw material:

- enzymatic hydrolysis of starch and non-starch polysaccharides with subsequent protein separation through centrifugation.

The following section presents a comprehensive outline of the methodologies and procedures employed in executing the aforementioned studies.

2.5.1. Oat protein extraction from oat flakes through enzymatic hydrolysis / *Auzu proteīna ekstrakcija no auzu pārslām ar fermentatīvās hidrolīzes palīdzību*

Treating starch with α-amylase

Oat flakes were mixed with prior heated water at a temperature of 80±1 °C wherein α-amylase was added in the amount of 0.05% by volume. Then, continuously stirring, oat flakes (room temperature) were added at a ratio of 1:10 by weight. The mixture was stirred periodically at intervals of about 30±2 s every 3 minutes with a hand mixer Promix (Phillips, Hungary) for 30 minutes while the temperature of hydrolysis was kept in the range of 75–80 °C. The hydrolysate was then separated by the Hereus Multifuge X3 (Thermo Fisher Scientific, Germany) at G-force 900 for 1 second to separate the fibre. The obtained clarified hydrolysate was then separated at G-force 4800 for 5 minutes. The separated protein biomass was washed with water at a ratio of 1 to 4 by weight. The washed protein biomass passed separation before the mentioned centrifuge at G-force 4800 for 5 minutes and then dried at 65±2 °C in the incubator B5745-5-M (AEG, Germany) for 24 hours. Dried oat protein was milled with the hammer mill LM 3100 Perten Instruments (Perkin Elmer, USA), sieve 0.8 mm. The separated fibre was subjected to drying in the incubator at a temperature of 65±2 °C for a duration of 24 hours. The obtained samples were coded as A1 for protein and AF1 for fibre. A graphical representation of the technological steps describing oat protein extraction hydrolysing starch by α-amylase is presented in Appendix 1.

Treating starch and non-starch polysaccharides with α-amylase in combination with complex enzymes

Oat flakes were mixed with prior heated water at a temperature of 60±1 °C wherein amylase and complex enzymes Grainzyme FL were added in the amount of 0.05% by volume each. Room-temperature oat flakes were added at a flakes-water ratio of 1:10 by weight while the water was continuously stirred. The mixture was stirred periodically at intervals of about 30 s every 3 min. with the hand mixer Promix (Phillips, Hungary) for 20±1 minutes. Then the temperature of the mixture was raised to 80±1 °C, keeping the stirring intervals at the same periodicity for the next 20±1 minutes. The next subsequent processing steps were identical to those outlined in Chapter 2.5.1. The obtained samples were coded as AX1 for protein and AXF1 for fibre.

A graphical representation of the procedural steps describing oat protein extraction treating oat flakes with α-amylase and complex enzymes is presented in Appendix 2.

2.5.2. Oat protein extraction from oat flakes in 0.1 *M* NaCl solution / *Auzu proteīna ekstrakcija no auzu pārslām 0.1 M NaCl šķīdumā*

Oat protein was extracted by the methods mentioned in Chapter 2.5.1, treating oat flakes with α-amylase and in combination with complex enzymes prior to separation at G-force 4800. NaCl was then added to the clarified hydrolysate up to 0.1 *M* solution, then stirred with the

hand mixer Promix (Phillips, Hungary) for 1 minute and kept at 75±2 °C in the incubator B5745-5-M (AEG, Germany) for 4 hours. After retention, the protein extraction was processed by the same methods as above, wherein α-amylase and combined enzymes of α-amylase and complex enzymes were used. The obtained protein samples were named as AR1 and AXR1 for protein wherein α-amylase was used as the only enzyme and in combination with complex enzymes, respectively. Schemes of the technological steps describing oat protein extraction, treating oat flakes with α-amylase and in combination with complex enzymes with subsequent retention, changing the ionic strength with NaCl, are presented in Appendix 3 and Appendix 4, respectively.

To investigate the timeframe of protein structure changes related to protein recovery, the procedural steps involving adding NaCl to clarified hydrolysate up to 0.1 *M* solution, then stirring with the mixer Promix (Phillips, Hungary) for 1 minute and kept at 75±2 °C in the incubator, replicated at discrete time intervals of 0 and 6 hours. The subsequent procedural steps remain consistent with the aforementioned protocol. This methodology was specifically applied to samples where starch hydrolysis employed α-amylase. Samples were named as AR1. As a point of reference, the samples that omitted the phase involving the addition of NaCl to the clarified hydrolysate were employed, and these were subsequently named as A1 for further identification.

2.5.3. Deriving oat protein concentrate from oat flour through the process of enzymatic hydrolysis / *Auzu proteīna koncentrāta ieguve no auzu miltiem fermentatīvās hidrolīzes procesā*

Obtaining oat protein concentrate by means of starch and non-starch polysaccharide enzymatic hydrolysis in fine oat flour

Water was heated to a temperature of 60±1 °C, then α-amylase and complex Grainzyme FL enzymes were added at a range of 0.1% by volume each. The flour and enzymes ratio was based on earlier authors' experiments. Room-temperature oat flour was added at a ratio of 1:10 by weight, continuously stirring with the Promix (Phillips, Hungary) hand mixer. The obtained mixture was periodically kept stirred (for 30 s every 3 minutes) with the Promix (Phillips, Hungary) hand mixer for 20 minutes at a temperature of 60 °C. Then the temperature was raised to 75±2 °C for 20 minutes, while stirring remained at the same interval rate of 30 s every 3 minutes. The obtained hydrolysate was cooled down to 25±1 °C and passed separation with the centrifuge Hereus Multifuge X3 (Thermo Fisher Scientific, Germany), at G-force 4400, for 4 minutes. The obtained biomass was washed with water at the ratio of 1:10 and repeatedly passed separation, wherein separation parameters remained the same as previously. The washed biomass was dried in the incubator B5745-5-M (AEG, Germany) at a temperature of 60±2 °C for 24 hours. The dried protein concentrate was cooled down to room temperature and passed through the hammer mill LM 3100 Perten Instruments (Perkin Elmer, USA), sieve 0.8 mm. The milled sample of oat protein concentrate (OC1) was collected in a plastic sealed container and kept at room temperature for further analysis and processing. The scheme illustrating the aforementioned steps is presented in Appendix 5.

Obtaining oat protein concentrate by means of starch enzymatic hydrolysis in oat flour

Water was heated to a temperature of 75±1 °C, then α-amylase enzymes were added at a range of 0.1% by volume. The flour and enzyme ratio was based on earlier authors' experiments. Room temperature oat flour was added at a ratio of 1:10 by weight, continuously stirring with the Promix (Phillips, Hungary) hand mixer. The obtained mixture was periodically stirred (30 s every 3 minutes) with the Promix (Phillips, Hungary) hand mixer for 40 minutes at a temperature of 75±2 °C. The following processing steps were the same as mentioned above for

oat protein extraction, hydrolysing starch with α-amylase and complex enzymes Grainzyme FL. The obtained protein samples were coded as OC1B for protein. A diagram representing the steps mentioned above is shown in Appendix 6.

2.5.4. Oat protein defatting / *Auzu proteīna attaukošana*

Protein concentrate derived from oat flour through enzymatic hydrolysis contains a substantial amount of lipids. Two methodologies were selected for the extraction of lipids, utilising ethanol and supercritical fluid CO_2.

Precipitation with ethanol

OC1 was mixed with ethanol at a ratio of 1:6 w/v, continuously stirring with the Promix (Phillips, Hungary) hand mixer for 30 s, then placed in sealed glass jars and kept in the incubator B5745-5-M (AEG, Germany) at 65±2 °C for 4 hours. The time and temperature of oil extraction using ethanol were based on earlier authors' experiments. Glass jars were shaken by hand at an interval of about 5 s every hour. After 4 hours the decant was drained. The precipitate, solid biomass, was repeatedly mixed with ethanol at a ratio of 1:3 w/v and stirred with the Promix (Phillips, Hungary) hand mixer for 30 s, and kept at 65±2 °C for 1 hour in the incubator. The resulting decant was carefully drained and the precipitated biomass was dried in the incubator at a temperature of 65±2 °C for 24 hours. The obtained oat protein concentrate defatted by ethanol (ODE1) was then naturally cooled down to room temperature, collected in a plastic sealed container and kept at room temperature. The scheme in Appendix 7 presents an illustration outlining the aforementioned steps.

Precipitation with ethanol in wet sample

To demonstrate the effect of ethanol purity on lipid extraction from proteinaceous biomass, the sample lipid extraction process was performed on a wet sample. The sample was prepared as described in Chapter 2.5.3, wherein oat flour was treated with solely α-amylase. The sample preparation covered the steps up to obtaining "washed protein biomass". Further, the sample underwent the following treatment. The obtained washed protein biomass was mixed with ethanol at a ratio of 1:3 w/v, continuously stirring with the Promix (Phillips, Hungary) hand mixer for 30 s, then placed in sealed glass jars and kept in the incubator B5745-5-M (AEG, Germany) at 50±2 °C for 30 minutes. Glass jars were shaken by hand at an interval of about 5 s every 10 minutes. After 30 minutes the sample passed separation with the centrifuge Hereus Multifuge X3 (Thermo Fisher Scientific, Germany) at G-force 4400, for 4 minutes. The obtained pellet was repeatedly washed by mixing ethanol and the pellet at a ratio of 1:3 w/v, continuously stirring with the Promix (Phillips, Hungary) hand mixer for 30 s followed by separation by centrifuge, applying the same parameters as above. The resulting supernatant was carefully drained and the precipitated biomass was dried in the incubator at a temperature of 65±2 °C for 24 hours. The obtained oat protein concentrate defatted by ethanol was then naturally cooled down to room temperature, collected in a plastic sealed container and kept at room temperature. The scheme in Appendix 8 displays a diagram illustrating the aforementioned steps.

Treating protein with SC-CO_2

OC1 was defatted by applying laboratory-scale SC–CO_2 equipment (SFE 1000, Faneks, Ltd, LV). The extraction chamber was filled with 130±1 g of oat protein concentrate. Extraction was performed with pure CO_2 at the following conditions, flow rate of CO_2 – 5.5 kg h^{-1}, extraction time 4.5 h, pressure in the range of 285–300 bar, temperature 50±1 °C. The mixture of yellowish dim oily components was collected in a separate vessel. The mass percentage of

extracted oil was calculated determining the oil content in dry matter of treated material before and after CO_2 extraction. The obtained sample of oat protein concentrate defatted by SC-CO_2 (ODC1) was collected in a plastic sealed bag and kept at room temperature. The scheme of defatting oat protein concentrate with SC-CO_2 was presented in Figure 2.1.

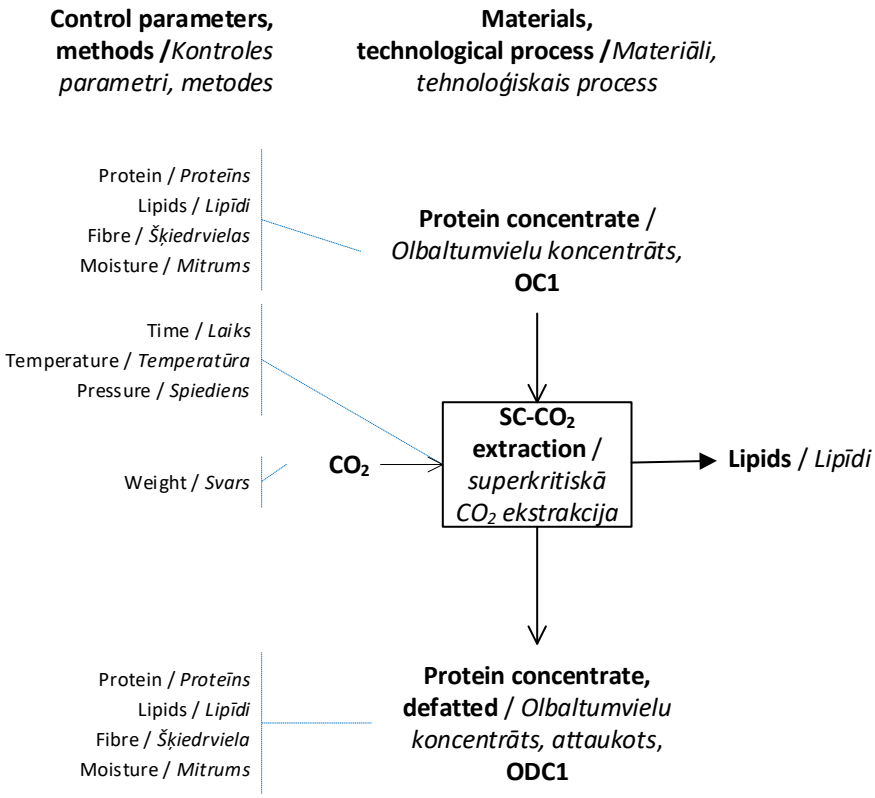


Figure 2.1. **Scheme of oat protein concentrate defatting with SC-CO_2**
Control parameters and methods describe measurements and applied analysis used for the particular process /
2.1 att. Auzu proteīna koncentrāta attaukošanas shēma ar SC-CO_2
Kontroles parametri un metodes apraksta konkrētajam procesam izmantotos mērījumus un pielietoto analīzi

2.6. Stage II of the research / *Pētījuma II posms*

Stage I focused on enzymatic protein extraction followed by defatting, with a primary emphasis on yield and concentration range. The current stage, however, directed its attention towards the examination of characteristics and functional properties associated with the acquired protein concentrates. This stage covered an analysis of the obtained materials, including their molecular mass and amino acid profile. Furthermore, it implied an investigation into the functional properties of the materials, particularly their protein solubility, foaming capacity, and water or oil holding capacity.

2.6.1. Molecular weight determination / *Molekulmasas noteikšana*

The sample powders were solubilised in 6 *M* urea and 2% SDS buffer. All samples were diluted to 4 mg mL^{-1} (scales TE-124S-OCE, Sartorius AG, Gottingen, Germany), then shaken for 1 minute with the heavy-duty vortex mixer VXHDDG (Ohaus, USA) at 2500 rpm. The suspensions were then shaken for 1.5 h with the environmental shaker incubator ES-20 (Biosan, Ltd, Latvia) at room temperature. The suspensions passed 15-minute centrifugation at G-force 2300 (centrifuge CM-6MT, Elmi Ltd, Latvia). The resulting supernatants of protein samples were collected and frozen at -18 °C.

Analyses were performed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using the Agilent Bioanalyzer 2100 capillary electrophoresis system (Agilent, USA) with the Agilent Protein 230 Kit (14–230 kDa sizing range). Briefly, aliquots of 4 µL unfrozen protein samples were mixed with 2 µL DDT denaturing solution, prepared according to Agilent protocols (3.50 Vol, -% of 1M DTT), spun for 15 s and then heated at 95±1 °C for 5 minutes, cooled down and diluted to 90 µL with deionised water. Ladder, Gel-Dye mix and destaining solution were prepared and loaded according to the Agilent assay protein protocols for the Bioanalyzer 2100.

2.6.2. Oat protein functional properties / *Auzu proteīna funkcionālās īpašības*

Protein solubility

The samples of oat protein concentrate, which were prior treated with ethanol and CO_2, were subjected to solubility treatment in aqueous solution at pH 3, 5, 7 and 9. The method of nitrogen solubility index was carried out as described by Morr et al. (1985), Sewada et al. (2014) with minor modification. One gram of protein concentrate treated with ethanol was dispersed in 0.1 *M* NaCl solution. The dispersions were adjusted to the specific pH value 3, 5, 7 and 9 with 0.1 *N* HCl or 0.1 *N* NaOH to a final volume of each dispersion of 50 mL, then continuously stirred with a magnetic stirrer for 2 hours at 25 °C. Values of pH were measured by means of the pH-meter Mettler Toledo Seven Compact (Mettler—Toledo GmbH, Germany) equipped with an Inlab Expert Pro-ISM pH-electrode. The dispersions were then separated by the centrifuge Hermle Z 206 A (Hermle Labortechnik GmbH, Germany) at G-force 4600 for 5 min. (25 °C). The supernatant was filtered through filtration paper (GOST 12026-76, FB-III-20, TU-2642-001-68085491-2011, ash content, no more than 0.00133%, filtration capacity < 26 seconds, bursting strength 5 kPa (Melior XXI, Ltd, RU). Nitrogen content in filtrates was determined by the Kjeldahl method. Nitrogen solubility index was calculated according to Equation 2.1.

$$\mathrm{NSI} = \frac{\text{Nitrogen in filtrate, \%} * \text{weight of solution (g)}}{\text{Nitrogen in dried sample, \%} * \text{weight of sample (g)}} * 100\ \% \qquad (2.1)$$

Nitrogen to protein conversion factor was set at 6.25.

Water/oil holding capacity

Water and oil binding capacity was determined according to the method described by Mirmoghtadaie et al. (2009) with minor modification. One gram of sample was dispersed in 10 g of commercial sunflower refined deodorised cooking oil or ultra-pure water and stirred with the heavy-duty vortex mixer VXHDDG (Ohaus, USA) for 1 minute at 25±2 °C, where the speed was set at 1200 rpm and 2500 rpm, for oil and water, respectively and left for 30 minutes. During rest time the samples were periodically shaken for 10 seconds every 10 min. with the heavy-duty vortex mixer VXHDDG (Ohaus, USA). Then the dispersions were separated by centrifuge Hermle Z 206 A (Hermle Labortechnik GmbH, Germany) at G-force 3000 for 5 minutes. The supernatant was poured and the pellet weighed. The oil and water holding capacities were determined expressing the amount of water and oil in grams retained per gram of protein concentrate, respectively.

Foaming capacity and foam stability

One gram of oat protein concentrate was mixed with 33 g of ultra-pure water at pH 7 in a 50 mL graduated glass cylinder, then continuously mixed for 30 min. with a magnetic stirrer. The dispersion was then continuously stirred with the high shear mixer T10 Ultra Turrax (IKA

Werke GmbH & Co. KG, Germany) for 5 min. The total volume of foamed mixtures was recorded at 5, 10, 30, 60 and 120 min. The method was adapted with slight modification as described by Mirmoghtadaie et al. (2009). Foaming capacity was calculated according to Equation 2.2.

$$\text{Foaming capacity} = \frac{VF}{Vi} * 100\ \% \tag{2.2}$$

where VF – a foam volume, mL;
Vi – an initial volume of the aliquot, mL.

2.7. Stage III of the research / *Pētījuma III posms*

Stage III of the study encompassed a study of the extrusion process applied to the protein concentrates that were characterised in the preceding stages. This stage focused on revealing the key characteristics and parameters of the extrusion system necessary for the production of wet extrudate. Alongside the analysis of the extrusion system, the research also presents comprehensive data on the properties of the obtained extrudate, including its textural attributes and colour. Furthermore, the oat protein extrudate was subjected to analysis under a Scanning Electron Microscope (SEM) revealing the longitudinal and cross-sectional images for analysis.

2.7.1. Protein extrusion / *Proteīna ekstrūzija*

The oat protein concentrate defatted by ethanol (ODE1) was mixed with pure water to the final content of dry solids of 55±1 % by weight. The water was dispersed on the top of the ODE1 and mixed with a spatula to ensure homogeneity of the distribution of raw materials. The blended material was left for half an hour at room temperature in an open container before the extrusion process.

To compare the extrusion parameters, soy protein concentrate was used, wherein the composition of raw material was described in Table 2.2. The preparation method of soy protein concentrate for extrusion was identical to that of ODE1, aiming to achieve a final dry solids content of 55±1% by weight before the extrusion process.

The blend of the prepared raw material was processed in a single screw extruder Extrusiometer L20 (Göttfert, Germany). The diameter of the installed screw was 20 mm, wherein the length-diameter ratio was L=25 D, and coefficient of the compression 1:1. The temperature for the 3-barrel heating zones was set at 90–110–130 °C, which were controlled by installed electric heaters and forced air cooling. The screw rotation speed was set at a constant speed of about 50 rpm to keep pressure in the range of 5–8 bar at the end of the barrel. The die providing a shear stress had a square 13×13 millimetre slit, and the feed rate was not calculated. The raw material was continuously provided, manually keeping the feed end always filled and pressed, ensuring that the raw material was sufficiently fed.

Some of the representative samples of the protein extrudates were sliced in tangential and cross sections and passed through the freeze drier for moisture elimination. The remaining samples were dried in the freeze drier FT33 (Armfield Ltd, UK) at –40±3 °C (in condenser chamber) and 6.4 Pa pressure without size reduction in the form obtained in the extrusion process. Freeze drying was performed for about 48±1 h until the moisture content in the samples reached 3.5±0.2%.

2.7.2. Colour analysis / *Krāsas analīze*

Colour measurements were performed for the initial untreated raw materials and extrudates. The extrudates were collected and measured within 30 min. after extrusion. The colour was determined using the colorimeter Colour Tec-PCM/PSM and software ColorSoft QCW (Accuracy Microsensors, Inc., Pittsford, NY, USA), evaluating the colour in the CIE

L*a*b system. The total colour difference (ΔE) was calculated according to Equation 2.3. (Kirse-Ozolina, Muizniece-Brasava, & Veipa, 2019; J. Zhang et al., 2020).

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2.3)$$

where ΔE – total colour difference;
L^*– colour intensity (light-dark) in extrudate;
L_0^* – colour intensity (light-dark) in the initial material;
a^* – green-red colour component in the extrudate;
a_0^* – green-red colour component in the initial material;
b^* – blue-yellow colour component in the extrudate;
b_0^* – blue-yellow colour component in the initial material.

2.7.3. Texture analysis / *Struktūras analīze*

The textural properties of extrudates were analysed by the TA-HD Plus texture analyser and data were generated by Exponent software (Stable Microsystems Ltd, Godalming, UK). For each sample of protein extrudate at least 5 measurements were completed. The cutting parameters: pre-test speed 1 mm s^{-1}; test speed 5 mm s^{-1}; post-test speed 10 mm s^{-1}; cutting distance of 20 mm into the extrudate sample, trigger force 0.049 N. The samples were cut into cross sections using an HDP/BSK probe (standard blade set with knife). Generated values of peak force (N), peak positive force (N) and positive area (N × s) were expressed as fracturability, hardness and toughness (Linly et al., 2021; Ojha et al., 2022), respectively.

2.7.4. Analysis of the microstructure of the obtained extrudate / *Iegūto ekstrudātu mikrostruktūras analīze*

The structure of the surface of the dried extrudate was analysed by a scanning electron microscope (Tescan MIRA3 XMU, Czech Republic) without any surface treatment required. The magnification, voltage, and segment value in microns were automatically reported and seen in each micrograph below. The focus location was chosen from the point of view of the best representative visibility of the sample.

2.8. Mathematical data processing / *Matemātiskā datu apstrāde*

Friedman rank sum test was applied, analysing the median differences among polar and one-way ANOVA for non-polar amino acid groups with the prior Shapiro-Wilk normality test. The T-test was applied for textural analysis. Data in tables and graphs is expressed as the mean ± standard deviation for at least three replications if it is not mentioned separately. ANOVA tests followed by Tuckey's HSD and a compact letter display were applied for the remaining analyses unless stated otherwise. Statistical analysis was conducted in R (R Core Team, 2022). Figures and data were processed using R packages (Graves, Piepho, & Dorai-Raj, 2023; Kassambara, 2023; Patil, 2021; Wickham et al., 2019). RStudio (RStudio Team, 2022) was used for the Integrated Development Environment for R.

3. RESULTS AND DISCUSSION / *REZULTĀTI UN DISKUSIJA*

3.1. Oat protein isolation / *Auzu proteīna izolēšana*

3.1.1. Protein isolation from whole oat flakes by enzymatic hydrolysis / *Proteīnu izolēšana no pilngraudu auzu pārslām ar fermentatīvās hidrolīzes palīdzību*

The present study comprises the data related to protein recovery from whole oat flakes through enzymatic extraction. Two approaches were studied: treating oat flakes with α-amylase for starch hydrolysis and applying two kinds of enzymes simultaneously, α-amylase and complex enzyme, which initiate the hydrolysis of starch and non-starch polysaccharides, respectively. Subsequently, protein separation was accomplished using centrifugal force. The obtained results provide information on the protein concentration, fat content, crude fibre content, and protein yield of the samples, as detailed in Table 3.1.

The extraction of protein was conducted in distinct batches, with each comprising approximately 400 g of the initial oat material. The dried samples were subsequently analysed to determine their protein concentrations, which ranged from 84.6% to 85.9% in dry matter (DM) by weight, for A1 and AX1, respectively.

Table 3.1. / *3.1. tabula*

Chemical characterisation of initial oat flakes and oat protein concentrates / *Sākotnējo auzu pārslu un auzu proteīna koncentrātu ķīmiskā sastāva raksturojums*

Sample / *Paraugs*	**Crude protein, in DM /** *Kopproteīns sausnā,* **g 100 g^{-1}**	**Fats, in DM /** *Koptauki, sausnā* **g 100 g^{-1}**	**Crude fibre, in DM /** *Kopējās šķiedrvielas sausnā,* **g 100 g^{-1}**	**Protein yield* /** *Proteīnu iznākums*, %*
FL1	17.56±0.03 d	5.7±0.11 c	2.1±0.10 b	-
A1	84.64±1.64 a	3.0±0.14 d	1.4±0.08 c	35.9±0.70 b
AX1	85.86±1.80 a	5.7±0.08 c	1.1±0.05 c	28.1±0.56 c
AF1	30.30±0.62 c	7.6±0.16 b	5.5±0.37 a	35.9±0.73 b
AXF1	39.36±0.76 b	9.0±0.40 a	5.2±0.34 a	47.6±1.02 a

* % of protein content in initial material. Data expressed as means ± standard deviations within the column not sharing any letter are significantly different by the ANOVA test at a 5% level of significance; FL1 – whole oat flakes; A1 – oat protein concentrate treated with α-amylase; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AF1 – oat fibre, treated with α-amylase; AXF1 – oat fibre, treated with α-amylase and complex enzymes / ** % no proteīna satura sākotnējā materiālā. Dati, kas izteikti kā vidējie ± standartnovirzes kolonnā, un kuriem nav kopīgi burti, būtiski atšķiras pēc ANOVA testa 5% nozīmīguma līmenī. FL1 – pilngraudu auzu pārslas; A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AF1 – auzu šķiedrvielas, apstrādāta ar α-amilāzi; AXF1 – auzu šķiedrvielas, apstrādāta ar α-amilāzi un kompleksiem enzīmiem*

The separation of the hydrolysate under low G-force conditions resulted in a significant redistribution of compounds in contrast to the original whole oat flakes. In addition to the separated fibre, the suspended solids comprised a substantial portion of the raw protein. When employing α-amylase as the sole enzyme, the method facilitated protein concentration into the fibre stream, reaching up to 30.3%. Another investigated method, involving the breakdown of non-starch polysaccharides, led to even higher levels of protein concentration. The depolymerisation of starch and non-starch polysaccharides in the hydrolysate weakened the attractive forces and reduced viscosity, resulting in a protein concentration increase of up to 39.4%. However, such a high protein concentration also caused protein redistribution. The recovery of protein in the fibre streams reached up to 35.9% and 47.6% when oat flakes were

treated with α-amylase alone and in combination with complex enzymes, respectively. On the other hand, the increase in protein produced a stream with relatively high concentration and yield which might further be used in applications requiring high fibre and protein content, such as plant-based protein products. However, according to Peterson (2011), the high protein concentration in fibre might also be considered as an associated material with aleurone and sub-aleurone layers, which are typically rich in protein.

Apart from protein redistribution, the separation process carried out at a relatively low G-force also had an impact on lipid concentration. Consequently, the protein concentrate composition of sample A1 contained approximately 3.0% lipids, whereas sample AX1 exhibited a lipid content of approximately 5.7%. It is possible to speculate that the increase in lipid content may be attributed to the depolymerisation of non-starch components, leading to the formation of lipid complexes within the oat flakes to some extent.

As expected, the fibre content was lower in samples subjected to treatment with complex enzymes. Consequently, the protein concentrate A1 demonstrated a higher fibre content (1.4% by weight) compared to AX1 (1.1% by weight), although the differences were not substantial and did not demonstrate significant variation.

The achieved protein concentration in the present study was comparable to or even higher than the concentrations reported in studies that employed alkaline extraction methods for protein extraction. For instance, it was documented that the utilisation of harsh alkaline protein solubilisation led to protein concentrations of up to 68.4% in DM, wherein the pH of the slurry was set to 12.1 (C. Y. Ma, 1983). Some modified techniques, when oat brans were employed as the raw material, involving enzyme pre-treatment, specifically using xylanase, α-amylase, glucoamylase, and cellulase, before the subsequent 2 *M* NaOH alkaline extraction, led to protein concentrations of up to 82.0% (Jodayree et al., 2012). In the study of Prosekov et al. (2018), it was demonstrated that treating oat brans with amyloglucosidase facilitated a protein concentration of up to 83.8%. However, the specific yield of this process was not reported.

In recent publications, novel approaches involving the introduction of protein-glutaminase followed by protein separation through ultrafiltration have demonstrated enhanced protein solubility. However, the resulting protein concentration reached a maximum of 52.4% (Immonen, Myllyviita, et al., 2021).

3.1.2. Ionic strength influence on proteins / *Jonu stipruma ietekme uz proteīniem*

The study focused on investigating the effects of ionic concentration changes in the solution on protein aggregation and its subsequent recovery from clarified hydrolysate. As mentioned earlier, when oat flakes were employed as the raw material, the protein recovery from clarified hydrolysate accounted for up to 35.9% by weight of the total protein. Based on current research findings, the existing limitations could presumably be associated with the protein molecular weight and water-protein interactions, impeding the efficiency of separation through centrifugation. The efficiency of the recovery by separation is subjected to various factors, encompassing particle properties and forces participating in particle-particle separation. It is generally believed that the aggregation of particles, particularly when similar particles aggregate and result in larger aggregate sizes, leads to significant differences in forces, thereby exerting a positive influence on the separation process (van Hee, Hoeben, van der Lans, & van der Wielen, 2006).

Thus, the hypothesis put forward suggested that the protein recovery efficiency could be linked to protein aggregation. The aforementioned hydrolysates, derived from oat flakes, underwent treatment using a sole α-amylase enzyme individually, as well as in combination with complex enzymes, followed by an additional process involving ionic shift. The content of Table 3.2 represents the outcomes pertaining to the concentration of protein and oil in samples that have undergone treatment with NaCl salt.

Table 3.2. / *3.2. tabula*

Chemical characterisation of oat protein concentrates after treatment with 0.1 *M* NaCl salt / *Auzu proteīna koncentrātu ķīmiskā sastāva raksturojums pēc apstrādes ar 0,1M NaCl*

Sample / *Paraugs*	**Crude protein, in DM /** *Kopproteīns sausnā,* **g 100 g^{-1}**	**Fats, in DM /** *Koptauki sausnā,* **g 100 g^{-1}**	**Protein yield* /** *Proteīnu iznākums*,* **%**
AR1	84.20±1.89 a	5.20±0.06 b	44.80±0.83 a
AXR1	86.46±2.23 a	6.30±0.20 a	33.10±0.51 b

* % of protein content in initial material. Different letters within a column indicate significant differences for each parameter ($p < 0.05$). AR1 – protein concentrate treated with α-amylase and NaCl; AXR1 – protein concentrate treated with α-amylase, complex enzymes and NaCl / **% no proteīna satura izejvielā. Dažādi burti kolonnā norāda uz būtiskām atšķirībām katram parametram ($p < 0.05$). AR1 – proteīna koncentrāts apstrādāts ar α-amilāzi un NaCl; AXR1 – proteīna koncentrāts apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl*

Additionally, the results presented in Table 3.2 demonstrate the efficiency of the protein recovery. The protein concentration in samples ranged from 84.2% to 86.5%, for AR1 and AXR1, respectively. The introduction of NaCl resulted in a protein yield increase of approximately 24.8% and 17.8% in samples where whole oat flakes were subjected to treatment with α-amylase alone and in combination with complex enzymes, respectively.

As can be seen in Figure 3.1, the protein recovery efficiency was increased significantly in both cases. However, as expected, the ionic shift has not significantly influenced the concentration of raw protein due to the limited number of impurities exposed to precipitation through centrifugation. On the other hand, it can be asserted that the retention step resulted in a greater oil concentration in the protein concentrates in both cases, as compared to the samples that did not undergo the retention process. As typically oil has not been noticed to be subjected to separation on the pellet side, it could be concluded that the ionic shift only promotes lipids binding to protein to a certain extent. Furthermore, it can be posited that protein aggregation is partially influenced by hydrophobic interactions between lipids and proteins, which play a critical role in the process of protein aggregation and also contribute to the stabilisation of protein-lipid complexes (Alzagtat & Alli, 2002).

Visual observation revealed that protein started to aggregate in the second hour after NaCl salt was introduced. Both samples, with the presence of complex enzymes and with sole α-amylase were performed in a similar way. Notably, visible protein association became apparent starting from the third hour and continued to develop, ultimately stabilising in the fourth hour without any subsequent improvement. The visual observation of protein aggregation is presented in Figure 3.2. Concurrently, the recovery efficiency displayed a time-dependent improvement, reaching a plateau in the fourth hour and exhibiting no significant further enhancement by the sixth hour. Protein recovery demonstrated improvement until the fourth hour, which corresponded to observable aggregation. Subsequently, further dynamics ceased (see Figure 3.3). However, such a finding contrasts with certain reported earlier observations related to the speed of plant protein aggregation.

The closest related study pertains to soy protein isolation, where the observed effect on retention time was found to be most noticeable within the initial 10 minutes; later protein dissociated into smaller fragments (W. Wang, Zhang, et al., 2021). Li and Xiong (2021) carried out a research study to investigate the influence of various ionic strength conditions on oat protein aggregation. Their investigation revealed a rapid protein aggregation under different ionic strength levels, with a retention time of approximately 20 minutes. Caution must be exercised when considering the dissimilarities, as the studied materials were prepared using different methods, specifically involving drying and subsequent rehydration, which inherently influences their subsequent behaviour in solutions.

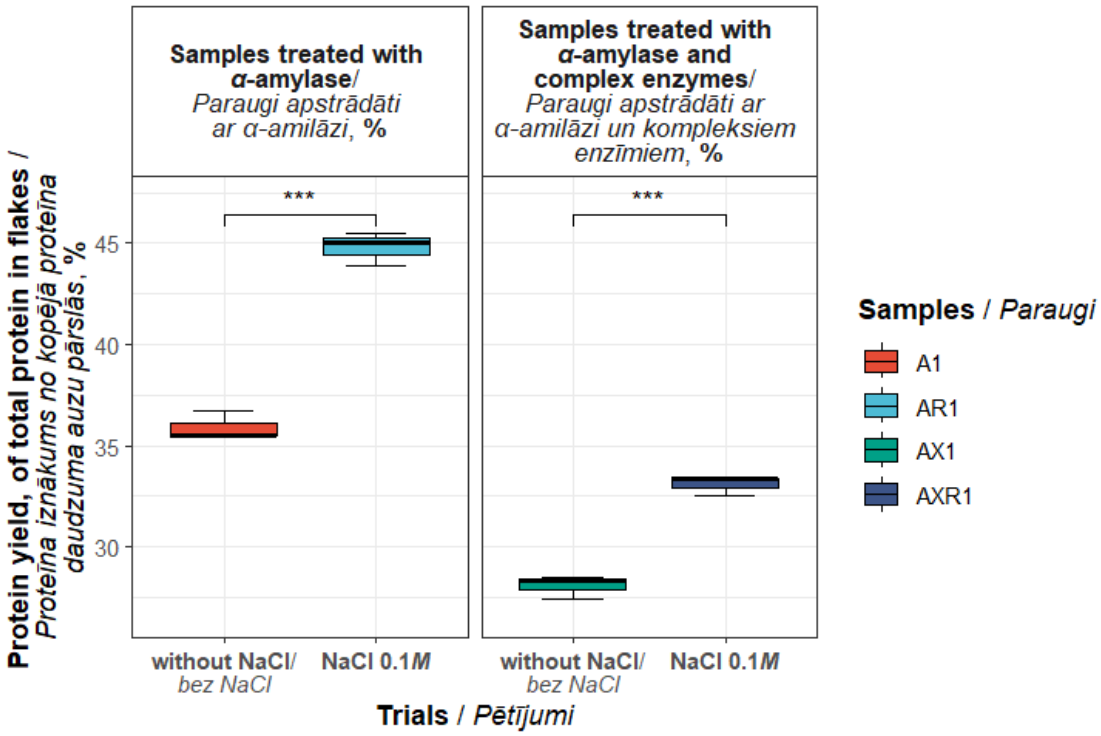


Figure 3.1. **Influence of 0.1 *M* NaCl solution on the efficiency of protein recovery in oat protein samples subjected to treatment with α-amylase alone and a combination of α-amylase and complex enzymes**

*** p ≤ 0.001; A1 – oat protein concentrate treated with α-amylase; AR1 – protein concentrate treated with α-amylase and NaCl; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – protein concentrate treated with α-amylase, complex enzymes and NaCl.

3.1. att. 0.1 M NaCl šķīduma ietekme uz proteīnu atgūšanas efektivitāti auzu proteīna paraugos, kas pakļauti apstrādei tikai ar α-amilāzi un α-amilāzes kombināciju ar kompleksiem enzīmiem

**** p ≤0.001; A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi un NaCl; AX1-auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl*

Figure 3.2. **Visual observation of oat protein aggregation immediately after preparation and incubation for 3 and 4 hours at 75 °C /**

3.2. att. Auzu proteīna agregācijas vizuāla novērošana uzreiz pēc sagatavošanas un pēc 3 un 4 stundu inkubācijas 75 °C temperatūrā

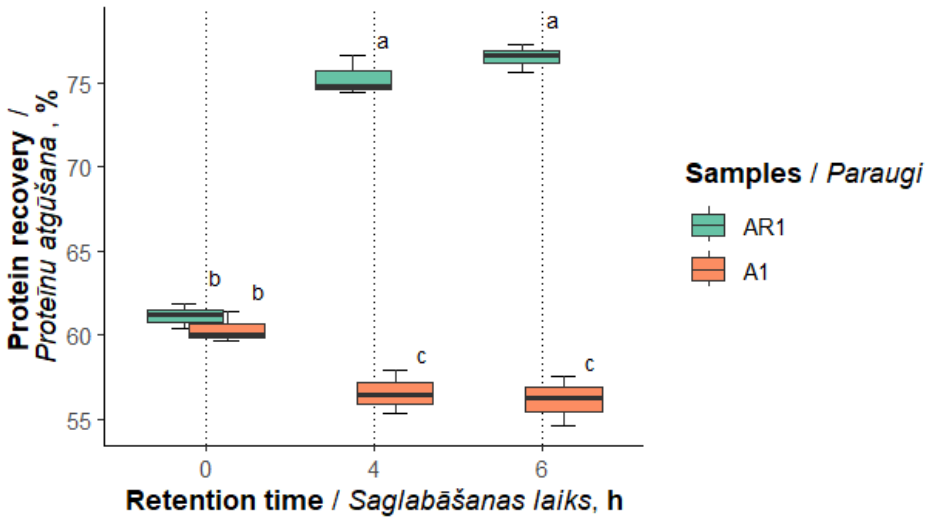


Figure 3.3. **Influence of 0.1 *M* NaCl solution on protein recovery from clarified hydrolysates, subjected to α-amylase treatment immediately after preparation and after 4 and 6 hours of incubation at 75 °C**

Means sharing a common letter do not differ significantly at a 5% level of significance

3.3. att. 0,1M NaCl šķīduma ietekme uz proteīnu ieguvi no dzidrinātiem hidrolizātiem, kas pakļauti α-amilāzes apstrādei tūlīt pēc sagatavošanas un pēc 4 un 6 stundu inkubācijas 75 °C temperatūrā

Vienādi burti parāda, ka starp paraugiem nav būtiskās atšķirības 5% būtiskuma līmenī

3.1.3. Protein isolation from fine oat flour / *Proteīnu ieguve no smalkā maluma auzu miltiem*

Our previous studies have shown that the separation of fibre resulted in a significant decrease in protein recovery when oat flakes were used as the raw material. As an alternative approach to protein recovery, one can consider utilising raw materials with reduced fibre content, achieved through the commercial milling process involving dry fibre separation by sieving. Oat flour utilised in the current investigation contained a substantially lower amount of fibre than the aforementioned oat flakes, with a measured proportion of 0.59% by weight in contrast to 2.10% by weight, respectively. Utilising flour as an initial raw material enabled the modification of processing steps, eliminating the need for low G-force fibre separation.

The protein extraction process wherein complex enzymes in combination with α-amylase were used was conducted in separate batches, starting with an initial amount of 2 000±1 g of flour. The resulting proteinaceous biomass in the underflow averaged 1039±15 g, and the subsequent second wash reduced the amount of biomass to 703±15 g, with dry solids accounting for approximately 33.0±3.0%. The dried sample counted protein at 63.05±1.3%, in the dry matter. The overall protein yield amounted to 77.0±3.0%, in comparison to the initial protein content present in the oat flour.

Exclusion of complex enzymes during the hydrolysis process resulted in a more viscous hydrolysate solution, consequently yielding a higher amount of intermediate proteinaceous biomass (+28.8% by wet weight) and washed protein biomass (+16.6% by wet weight) in comparison to the procedure involving the hydrolysis of non-starch polysaccharides. However, the considerable increase in intermediate products was attributed to increased moisture content, which exhibited an average of approximately 78.1% for the washed biomass. Consequently, this yielded a lower protein concentration in the dried sample (OC1B), which averaged at 59.9±2.0%, accompanied by a crude oil content of 14.61%, and an expected increase in fibre content by 1.8%. Findings that characterise the analysed samples are provided in Table 3.3.

Table 3.3. / *3.3. tabula*

Chemical characterisation of oat flour and oat protein concentrates obtained from oat flour, g 100 g^{-1} in DM / *Auzu miltu un no auzu miltiem iegūto auzu proteīna koncentrātu ķīmiskā sastāva raksturojums, g 100 g^{-1} sausnā*

Sample / *Paraugs*	**Crude protein /** *Kopproteīns*, **(N × 6.25)**	**Fats /** *Koptauki*	**Crude fibre /** *Kopējās šķiedrvielas*
Oat flour / *Auzu milti* (OF1)	10.44±0.32c	6.21±0.28c	0.59±0.21c
Protein concentrate / *Proteīna koncentrāts* (OC1)	63.05±1.30a	20.55±0.40a	1.34±0.07b
Protein concentrate /*Proteīna koncentrāts (OC1B)*	59.90±1.48b	14.61±0.82b	1.80±0.13a

OC1 – oat protein concentrate treated with α-amylase and complex enzymes; OC1B – oat concentrate treated with α-amylase. Data expressed as means ± standard deviations within the column not sharing any letter are significantly different by the ANOVA test at a 5% level of significance / *OC1 – auzu proteīna koncentrāts, kas apstrādāts ar α-amilāzi un kompleksajiem enzīmiem; OC1B – auzu proteīna koncentrāts, kas apstrādāts ar α-amilāzi. Dati, kas izteikti kā vidējie ± standartnovirzes kolonnā, kuriem nav kopīgi burti, būtiski atšķiras ar ANOVA testu 5% nozīmīguma līmenī*

The decreased protein concentration and increased moisture content in the washed biomass of OC1B led to a substantial reduction in protein recovery as compared to sample OC1, as illustrated in Figure 3.4. The considerable protein yield in both samples could be attributed to the distinctive properties of oat globulin, particularly its significant insolubility in neutral pH aqueous solutions, with further elaboration found in section 3.2. Furthermore, it might be assumed that the relatively high protein concentration led to the formation of insoluble protein aggregates, which could be attributed to its increased tendency for intermolecular bonding with surrounding proteins in their vicinity (G. Liu et al., 2009). In addition, the protein's surface hydrophobicity can be recognised as a supplementary factor promoting protein recovery, given its inverse correlation with protein solubility in aqueous solutions (Jing, Yang, & Zhang, 2016).

It is worth noting the fact that oat protein was extracted from oat flour, which underwent a pre-processing step where a significant portion of non-endosperm crop tissues were removed. This reduction minimises the loss of yield since a significant portion of water-soluble albumins are located in the embryonic axis and scutellum (Peterson, 2011), and regions that are typically subjected to separation. Furthermore, the aleurone layer, which is a major location of enzymes (Hu, Wei, Ren, & Zhao, 2009; S. S. Miller & Fulcher, 2011), was also removed to some extent from the oat flour during processing. As a result, the protein fraction subjected to subsequent analysis can be considered as predominantly composed solely of the water-insoluble globulin fraction.

Initially, oat flour comprised 6.21±0.28% wt. of lipids in raw material, although the concentration of oil in the oat protein concentrate increased up to 20.55±0.40% wt. and 14.61±0.82% wt. (see Table 3.3), depending on the enzymes utilised for hydrolysis. Considering the protein recovery process, which led to a significant accumulation of oil in the protein fraction, the resulting oat protein concentrates contained up to 42.6% of the total lipid content when a combination of α-amylase and complex enzymes were employed.

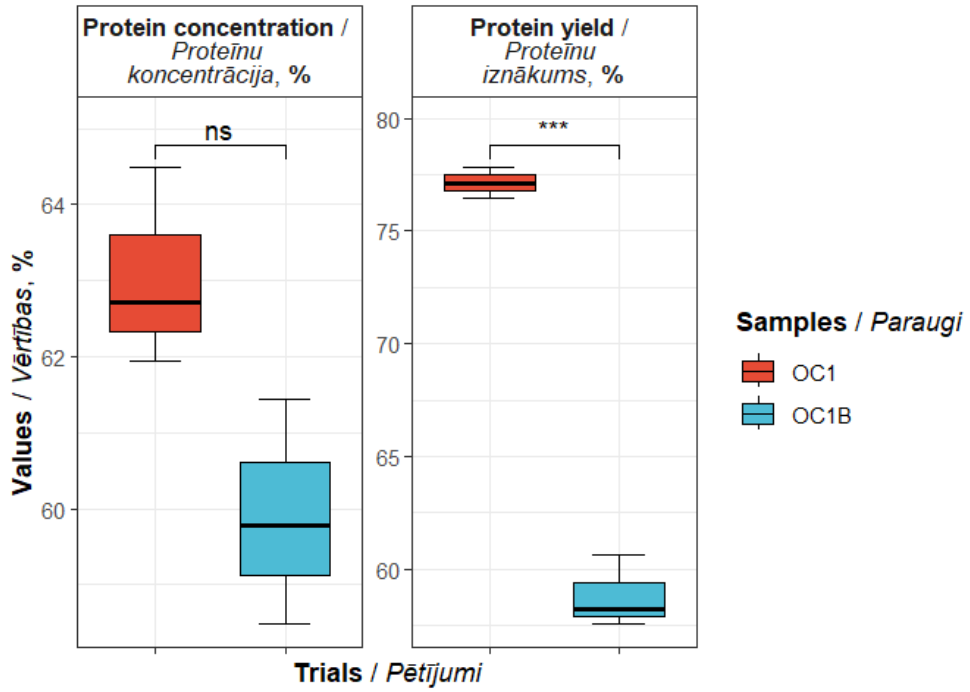


Figure 3.4. **Influence of enzymatic hydrolysis of non-starch polysaccharides on protein concentration and yield from oat flour, %**

ns: p > 0.05, *** p ≤ 0.001. OC1 – oat protein concentrate treated with α-amylase and complex enzymes; OC1B – oat concentrate treated with α-amylase

3.4. att. Cieti nesaturošo polisaharīdu fermentatīvās hidrolīzes ietekme uz proteīnu koncentrāciju un iznākumu no auzu miltiem, %

*ns: p > 0.05, *** p ≤0.001. OC1 – auzu proteīna koncentrāts, kas apstrādāts ar α-amilāzi un kompleksajiem enzīmiem; OC1B – auzu koncentrāts, kas apstrādāts ar α-amilāzi*

The study highlighted the critical role of media preparation during the hydrolysis step and its influence on the protein's ability to accumulate lipids during the separation process. Oat hydrolysate that underwent enzymatic treatment without affecting non-starch polysaccharides exhibited a significantly lower concentration of lipids in the protein fraction. Despite the lower degree of protein concentration achieved, the protein-lipid ratio remained consistently lower. This suggests that the interaction between proteins and lipids may involve several mechanisms, with a higher content of protein resulting in a higher binding of lipids. Figure 3.5 compares the concentration of lipids in the protein fractions and their accumulated percentage of total lipids found in native oat flour.

Conversely, the absence of enzymes capable of hydrolysing non-starch polysaccharides resulted in a significantly higher viscosity of the hydrolysate. This increased viscosity posed a constraint on the separation of proteins, causing them to remain in the supernatant phase along with the bound lipids.

The elevated concentration of lipids in the samples of the oat protein concentrates can be attributed to the substantial presence of non-polar oat lipids, which typically range from 65% to 90% of the total lipid content (Doehlert, Moreau, Welti, Roth, & McMullen, 2010). While the polar ends of lipids are bound to protein through the hydrophilic bonds, the non-polar ends interact with hydrophobic bonds. These non-polar lipids are generally not extractable with water without undergoing specific pre-treatment methods. Furthermore, the interactions between protein and lipids in an aqueous medium transform the protein structure to a certain extent by decreasing the intramolecular hydrophobic bonds, facilitating protein unfolding at the oil-water interface. Due to unfolding, the exposed protein reactive amino acids form hydrophobic bonds with neighbouring proteins. Thus, protein lipid hydrophobic interactions potentially influence protein aggregation (Alzagtat & Alli, 2002).

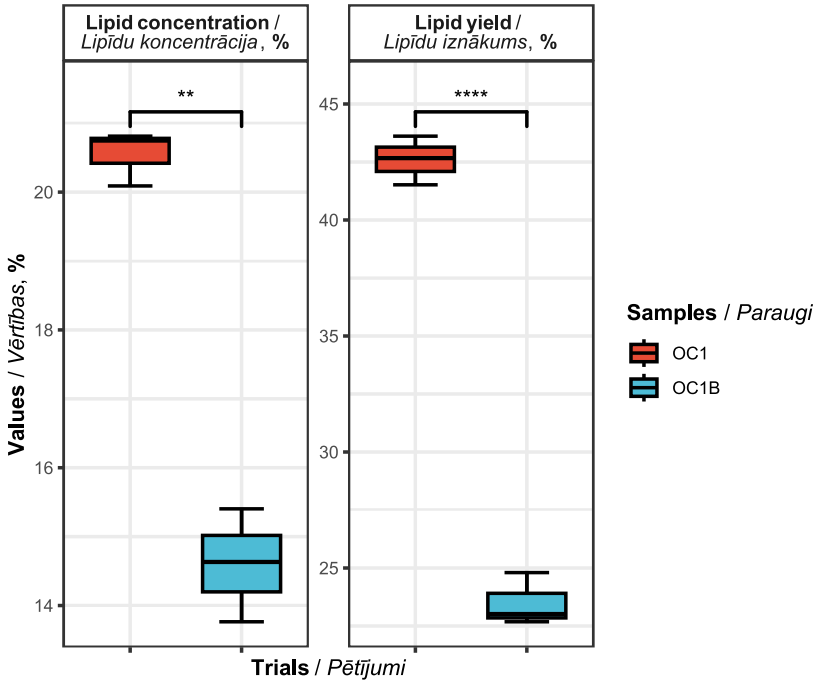


Figure 3.5. **Impact of enzymatic degradation of non-starch polysaccharides on lipid concentration and yield within protein samples derived from oat flour, %**

**p ≤ 0.01, ****p ≤ 0.0001. OC1 – oat protein concentrate treated with α-amylase and complex enzymes; OC1B – oat concentrate treated with α-amylase

3.5. att. Cieti nesaturošo polisaharīdu fermentatīvās noārdīšanās ietekme uz lipīdu koncentrāciju un iznākumu proteīna paraugos, kas iegūti no auzu miltiem, %

***p ≤0.01, ****p ≤0.0001. OC1 – auzu proteīna koncentrāts, kas apstrādāts ar α-amilāzi un kompleksajiem enzīmiem; OC1B – auzu koncentrāts, kas apstrādāts ar α-amilāzi*

3.1.4. Protein defatting / *Proteīnu attaukošana*

In contrast to oat protein concentrates derived from whole oat flakes, the protein concentrate obtained from oat flour had a comparatively higher lipid content, assuming the subsequent removal of lipids. Two distinct approaches were employed for lipid extraction from dried samples: precipitation utilising ethanol and extraction utilising supercritical fluid CO_2. Furthermore, the study investigated the extraction of lipids from wet biomass utilising ethanol, eliminating the need to employ the drying step.

The experiment was carried out by treating oat protein concentrate OC1 with ethanol. The oil content in the sample was reduced from 20.6% wt. to about 4.9% wt. Two-step extraction was performed. First, the main step involved subjecting the protein concentrate to 65 °C treatment for 4 hours (the period of time was determined experimentally) to remove the major part of the lipids. The second washing step wherein partially defatted protein concentrate was repeatedly washed with ethanol, also resulted in a visible change in the colour of the decant, indicating the presence of remaining lipids after the initial ethanol treatment. Removing spoiling lipids from the protein concentrate increased the protein concentration in the sample, which reached up to 78.18±1.93% wt. After the decantation process, the extraction of lipids using ethanol yielded a proteinaceous biomass with a powdery consistency, displaying a lack of stickiness. Attempts to enhance the density of the pellet through centrifugation did not yield favourable outcomes.

An even greater effect on lipid extraction was achieved when lipids were extracted by means of supercritical fluid CO_2. The concentration of lipids in the dried sample was reduced up to 3.50%. It could be speculated that the remaining oil in the protein concentrate contained a higher ratio of non-polar lipids than the initial material. During the protein concentration steps

the observable substantial amount of yellow liquid on the top of the supernatant was removed. It could be identified as a lipid fraction. Considering the fact that the hydrolysed material did not contain any solvent other than water, it can be speculated that the majority of the removed lipids during the separation process were likely polar lipids. This would lead to an increased concentration of non-polar lipids in the pellet and subsequently transferred to the extraction, resulting in higher levels of non-polar lipids. This might explain the observation of a higher extractability of oil in the supercritical system, despite the consideration of CO_2 as a gas for extracting presumably non-polar lipids. It is generally acknowledged that the further reduction of lipids is achievable when treating materials comprising lipids. In such cases, the addition of co-solvents, such as ethanol, to the CO_2 system is often considered to enhance extractability (Rad et al., 2019). Table 3.4 summarises the results of lipid extraction by ethanol and CO_2.

Table 3.4. / *3.4. tabula*

Chemical characterisation of oat protein samples after defatting by ethanol and supercritical fluid CO_2, g 100 g^{-1} in DM / *Auzu proteīna paraugu ķīmiskā sastāva raksturojums pēc attaukošanas ar etanolu un superkritisko šķidrumu CO_2, g 100 g^{-1} sausnā*

Sample / *Paraugs*	**Crude protein /** *Kopproteīns*, **(N × 6.25)**	**Fats /** *Koptauki*
Defatted by ethanol / *Attaukots ar spirtu* (ODE1)	78.18±1.93a	4.88±0.01a
Defatted by SC-CO_2 / *Attaukots ar SC-CO_2* (ODC1)	77.39±1.58a	3.48±0.11b

Data expressed as means ± standard deviations within the column not sharing any letter are significantly different by t-test at a 5% level of significance */ Dati, kas izteikti kā vidējie ± standartnovirzes kolonnā, kuriem nav kopīgi burti būtiski atšķiras t-testa 5% nozīmīguma līmenī*

The conducted investigation was based on the assumption that lipid extraction is carried out on proteinaceous materials in a dry state. However, eliminating the drying step would substantially improve the technological process in terms of capital and operational cost. To study the extraction rate from wet biomass an experiment was carried out wherein wet biomass was treated with ethanol.

The ethanol – dry biomass ratio was kept in the range of 3 to 1. The lipid content was reduced from the initial 14.6% to 13.1%. However, in spite of the statistically significant findings, the extracted lipids yielded a negligible amount. It was proposed that high levels of water in the solvent form a molecular cluster, which originates from a variety of hydrogen-bonding configurations between ethanol and water, subsequently reducing the transfer of soluble substances (Da Costa Rodrigues & Oliveira, 2010). Ethanol's inability to diffuse lipids to the bulk solution was obvious. Figure 3.6 provides a comparison of the results obtained from lipid extraction using ethanol, illustrating the differences between the extraction from dry and wet samples. The obtained results were consistent with previous published findings that demonstrated a decrease in the efficiency of ethanol extraction as the moisture content increased (Capellini et al., 2017; Da Costa Rodrigues & Oliveira, 2010; Kwiatkowski & Cheryan, 2002). Despite the attempt to extract lipids from wet biomass, the outcomes were relatively unsatisfactory, offering limited potential for further analysis.

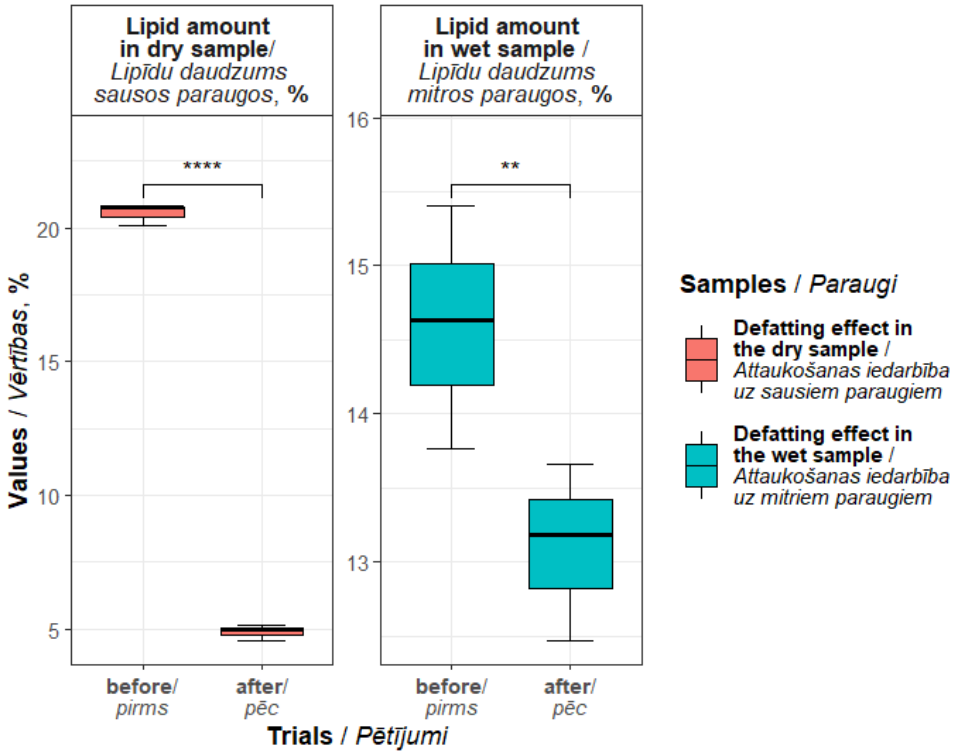


Figure 3.6. **Defatting effect on wet and dry oat protein samples, % /**
3.6. att. Attaukošanas iedarbība uz mitriem un sausiem auzu proteīna paraugiem, %
***p ≤ 0.01, ****p ≤ 0.0001*

Summary of Chapter 3.1 / *3.1. nodaļas kopsavilkums*

The study demonstrated that oat protein could be isolated and concentrated through enzymatic hydrolysis of starch, resulting in protein levels comparable to those found in commercial protein isolates (Gorissen et al., 2018). However, the production of protein concentrates originating from oat flakes, without suspended solids, led to a relatively low yield of protein recovery. Furthermore, the introduction of complex enzymes further decreased protein recovery to an even lower extent as the depolymerisation of starch and non-starch polysaccharides in the hydrolysate weakened attractive forces and reduced the viscosity of the hydrolysate. Shifting the ionic strength of the solution with NaCl significantly improved protein recovery by promoting protein aggregation and subsequent sedimentation. The study utilised oat flour to produce a lipid-rich protein concentrate. This concentrate underwent lipid extraction using ethanol and supercritical fluid CO_2. Both extraction methods, when applied to dried proteinous biomass, yielded consistent results, reducing the lipid content to below 5.0% by weight. However, attempts to extract lipids from wet proteinous biomass using ethanol yielded unsatisfactory results.

Pētījums parādīja, ka auzu proteīnu var izolēt un koncentrēt, izmantojot cietes fermentatīvo hidrolīzi, tādējādi iegūstot proteīnu daudzumu, kas ir salīdzināms ar proteīnu daudzumu rūpnieciskos proteīnu izolātos (Gorissen et al., 2018). Tomēr, ražojot proteīna koncentrātus no auzu pārslām bez suspendētām cietvielām, proteīna atgūstamība bija salīdzinoši zema. Turklāt, ieviešot kompleksos fermentus, proteīna atgūstamība samazinājās vēl vairāk, jo hidrolizātā esošās cietes un cieti nesaturošo polisaharīdu depolimerizācija vājināja pievilkšanās spēkus un samazināja hidrolizāta viskozitāti. Šķīduma jonu stipruma maiņa ar NaCl ievērojami uzlaboja proteīna atgūšanu, veicinot proteīna agregāciju un sekojošu nogulsnēšanu. Pētījumā tika izmantoti auzu milti, lai iegūtu taukiem bagātu proteīna koncentrātu. Šim koncentrātam veica tauku ekstrakciju, izmantojot etanolu un superkritisko

CO_2 šķidrumu. Abas ekstrakcijas metodes, ko piemēroja kaltētai proteīnu biomasai, deva vienādus rezultātus, samazinot tauku saturu zem 5,0% no masas. Tomēr mēģinājumi ekstrahēt taukus no mitras proteīnu biomasas, izmantojot etanolu, deva neapmierinošus rezultātus.

3.2. Oat protein characterisation and its functional properties / *Auzu proteīna raksturojums un funkcionālās īpašības*

The objective of the current investigation was to assess the functionality of oat protein concentrates obtained in earlier research. As protein extraction underwent fractioning by separation, the expected change of amino acid profiles in protein concentrates has been determined and compared to FAO (2007) recommendations. Furthermore, functional properties of proteins such as solubility, foamability, and liquid-holding capacity were investigated. The samples were classified according to the initial raw materials used and the methods employed for protein isolation. The samples were specifically categorised into two groups: those derived from oat flakes without suspended solids, and those derived from oat flour where suspended solids were concentrated alongside the protein.

3.2.1. Oat protein derived from whole oat flakes / *Auzu proteīns, kas iegūts no pilngraudu auzu pārslām*

Amino acids

Amino acid compositions of the examined samples are presented in Table 3.5. It is believed that the observation of changes in the amino acid composition of treated products can provide insights into the degree of modification they have undergone (Z. Wang et al., 2018). In the present study, the amino acid profiles among the samples demonstrated a relatively similar pattern. Comparing AR1 to A1, a minor decrease of approximately 10% in cysteine and around 8% in methionine were observed. Conversely, slight increases of approximately 7% in histidine and approximately 8% in isoleucine were detected in AR1 compared to A1. The manipulation of ionic strength did not significantly affect the amino acid content in samples treated with complex enzymes. The amino acid levels in these samples were relatively consistent, with variations within a range of 3% for certain amino acids such as histidine and isoleucine. Other changes in amino acid content between AX1 and AXR1 were even at a lower extent. A noticeable prevalence of glutamic acid, which is commonly found in oat crops, was observed when comparing the redistribution of individual amino acids.

Table 3.5. / *3.5. tabula*

Amino acid compositions in oat protein samples derived from oat flakes, g 100 g^{-1} of total protein / *Aminoskābju sastāvs auzu proteīna paraugos, kas iegūti no auzu pārslām, g 100 g^{-1} no kopējā proteīna*

Name / *Nosaukums*	**Sample /** *Paraugs*						
	FL1	**A1**	**AR1**	**AX1**	**AXR1**	**AF1**	**AXF1**
Ala	3.77±0.13b	3.77±0.05b	3.80±0.1b	3.83±0.05b	3.83±0.05b	4.48±0.14a	4.25±0.11a
Arg	6.55±0.17d	7.08±0.19cd	7.22±0.08c	7.14±0.16c	7.16±0.34c	11.17±0.21a	7.83±0.15b
Asp	7.54±0.28a	7.33±0.33a	7.57±0.2a	7.33±0.17a	7.44±0.2a	7.45±0.17a	7.61±0.32a
Cys	2.23±0.05c	2.83±0.12a	2.58±0.08b	2.82±0.03a	2.85±0.03a	2.18±0.09c	2.4±0.12bc
Phe	5.07±0.18c	5.90±0.08b	5.86±0.22b	5.88±0.06b	5.99±0.07ab	5.12±0.25c	6.4±0.17a
Gly	4.39±0.14bc	4.12±0.14c	4.32±0.19c	4.41±0.19bc	4.39±0.12bc	5.01±0.15a	4.77±0.08ab
Glu	19.91±0.42b	23.84±0.65a	23.85±0.79a	23.55±0.31a	23.44±0.47a	18.7±0.3b	19.71±0.76b
His	2.29±0.05c	2.40±0.04bc	2.58±0.12ab	2.46±0.07bc	2.54±0.05b	2.44±0.1bc	2.76±0.08a
Ile	3.59±0.16c	4.05±0.06b	4.38±0.14a	4.25±0.13ab	4.39±0.06a	4.01±0.06b	4.14±0.15ab
Leu	7.23±0.13b	8.46±0.17a	8.51±0.19a	8.49±0.1a	8.47±0.36a	7.45±0.3b	7.66±0.17b

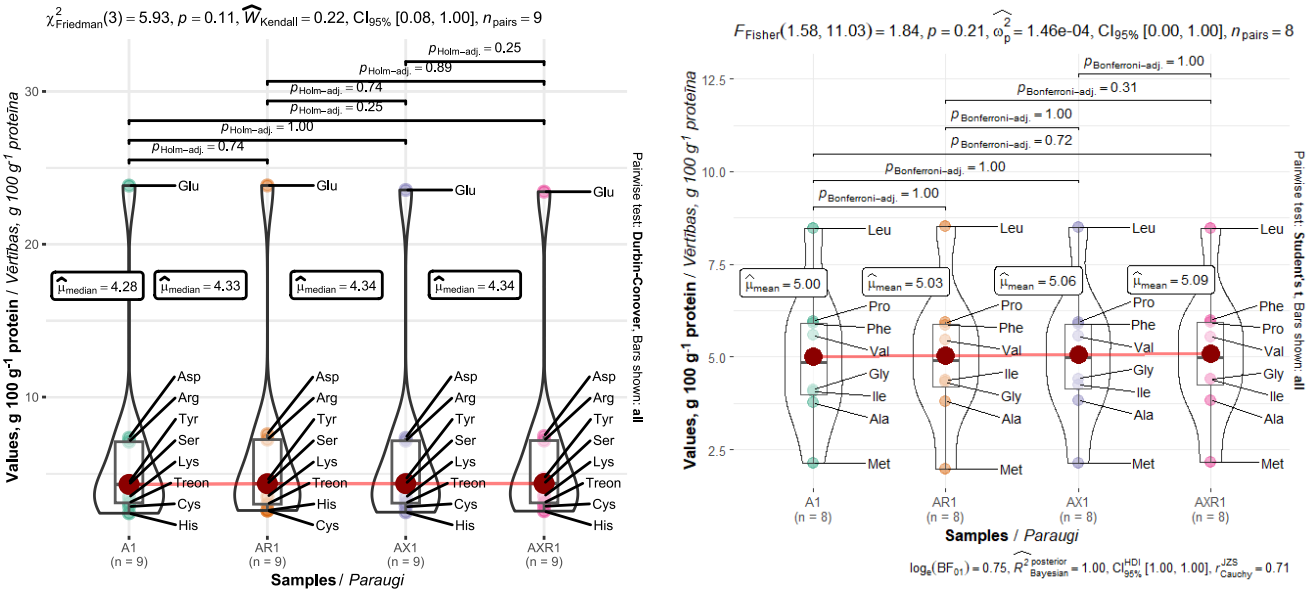
Continuation of Table 3.5. / *3.5. tabulas turpinājums*

Name / *Nosaukums*	**Sample /** *Paraugs*						
	FL1	**A1**	**AR1**	**AX1**	**AXR1**	**AF1**	**AXF1**
Lys	3.71±0.05bc	3.45±0.08d	3.48±0.06cd	3.44±0.05d	3.51±0.16cd	4.12±0.04a	3.78±0.11b
Met	1.67±0.08c	2.14±0.02a	1.99±0.04ab	2.14±0.08a	2.16±0.06a	1.65±0.08c	1.82±0.05bc
Pro	6.18±0.14a	5.96±0.15ab	5.93±0.26ab	5.94±0.25ab	5.93±0.11ab	5.48±0.25b	5.6±0.16b
Ser	4.08±0.05a	4.28±0.17a	4.33±0.15a	4.34±0.13a	4.34±0.17a	4.41±0.13a	4.47±0.2a
Tyr	3.59±0.12b	4.41±0.2a	4.54±0.12a	4.61±0.2a	4.68±0.07a	3.87±0.12b	4.69±0.16a
Thr	3.09±0.08b	3.08±0.14b	2.99±0.12b	3.06±0.1b	3.08±0.05b	3.44±0.07a	2.92±0.04b
Val	5.25±0.26a	5.59±0.11a	5.45±0.08a	5.56±0.2a	5.55±0.21a	5.37±0.17a	5.35±0.07a

Data expressed as means ± standard deviations within the row not sharing any letter are significantly different by the ANOVA test at a 5% level of significance. FL1 – whole oat flakes; A1 – oat protein concentrate treated with α-amylase; AR1 – protein concentrate treated with α-amylase; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – protein concentrate treated with α-amylase, complex enzymes and NaCl; AF1 – oat fibre, treated with α-amylase; AXF1 – oat fibre, treated with α-amylase and complex enzymes / *Dati, kas izteikti kā vidējie ± standartnovirzes rindā, kuriem nav kopīgi burti, būtiski atšķiras ar ANOVA testu 5% nozīmīguma līmenī. FL1 – pilngraudu auzu pārslas; A1 – auzu proteīna koncentrāts apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts apstrādāts ar α-amilāzi; AX1 – auzu proteīna koncentrāts apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – proteīna koncentrāts apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl; AF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi; AXF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi un kompleksiem enzīmiem*

The interaction between proteins and solvents, such as water, relies on the participation of functional groups or peptide bonds within an individual amino acid (Franks, Eagland, & Lumry, 1975). Electrostatic interactions with polar and charged groups, which are influenced by the presence of chaotropic salts, impact the hydrophobic interactions that contribute to protein unfolding (C. Y. Ma, Rout, Chan, & Phillips, 2000). Exposed groups play a crucial role in protein-protein interactions induced by salt. The albumin fraction typically exhibits a higher proportion of polar amino acids, while oat globulins are associated with an increased amount of non-polar amino acids (Jing et al., 2016). The protein aggregation observed upon modifying the ionic strength of the solution suggests that proteins from various fractions may have interacted. To investigate this in more detail, analysing the data by categorising amino acids according to their polarity, could help to ascertain if the protein aggregation affected the protein's origin. To examine this further, amino acids were categorised based on their polarity. However, the classification did not uncover any significant differences among the various groups. Figure 3.7 presents the quantitative measurements of amino acids grouped according to their polarity. The lack of noticeable changes in the content of amino acids indicates that the modification in ionic conditions did not have a substantial impact on the protein composition within the protein concentrate.

Although the protein concentrates displayed comparable amino acid profiles, the distribution of amino acids among the oat flakes and their derivatives obtained through the process, specifically in the oat protein concentrates and oat fibre fractions, exhibited notable variation. Figure 3.8 represents the percentage change in amino acid concentration of samples treated solely with α-amylase compared to the initial raw material.



(a) **(b)**

Figure 3.7. **Amino acid redistribution in (a) polar and (b) non-polar groups among the samples A1, AR1, AX1, AXR1, values, mean, g 100 g^{-1} protein**

3.7. att. Aminoskābju sadalījums (a) polārajās un (b) nepolārajās grupās starp paraugiem A1, AR1, AX1, AXR1, vērtības, vidējās, g 100 g^{-1} proteīna

FL1 – whole oat flakes; A1 – oat protein concentrate treated with α-amylase; AR1 – oat protein concentrate treated with α-amylase; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – oat protein concentrate treated with α-amylase, complex enzymes and NaCl; AF1 – oat fibre, treated with α-amylase; AXF1 – oat fibre, treated with α-amylase and complex enzymes / *FL1 – pilngraudu auzu pārslas; A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl; AF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi; AXF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi un kompleksiem enzīmiem*

A considerable increase in the concentration of cysteine, methionine, and tyrosine was observed in the protein concentrates. The most significant change occurred in arginine, with a substantial concentration increase observed in the oat fibre stream. An increased amount of arginine in the bran fraction was also observed by Ma (1983). Arginine prevails in 7S fractions of oat globulin (Burgess et al., 1983), although this association might be subject to some uncertainty assuming the challenging sedimentation of 7S at low-speed G-force, as operated in the present study. Lysine, which is considered a limiting amino acid in oat proteins, demonstrated a decrease in the concentrated protein, although it increased in the fibre stream. Furthermore, the decrease in proline was observed in all samples treated with sole α-amylase. The increased solution's ionic strength led to a decrease in the content of cysteine and methionine in AR1 compared to A1. However, AR1 exhibited higher levels of histidine and isoleucine than A1 did.

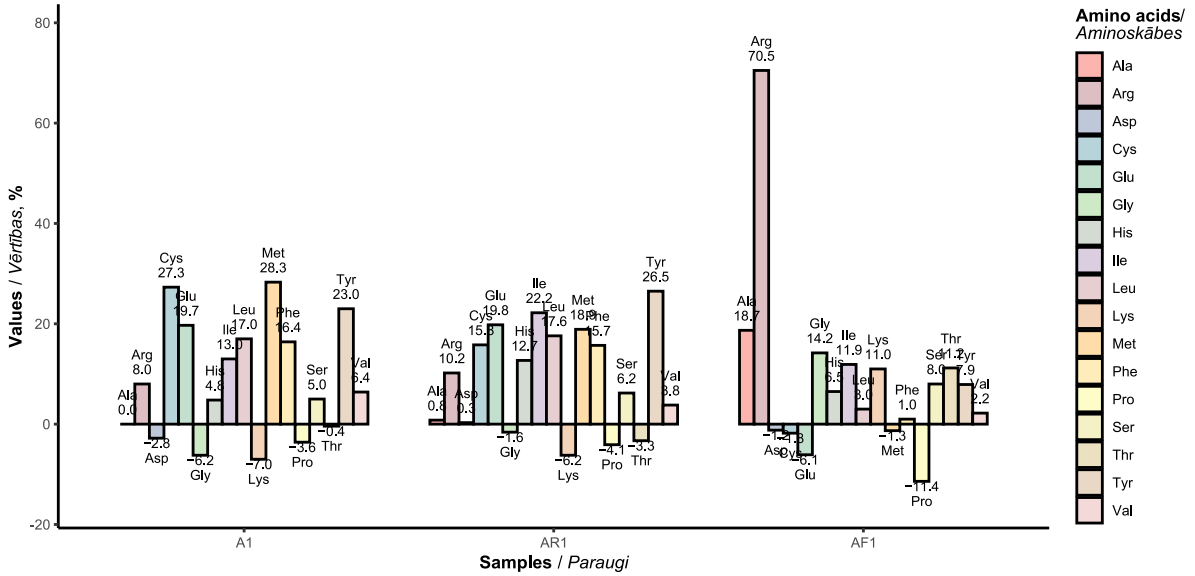


Figure 3.8. **Percentage shift in amino acid quantities observed in samples A1, AF1, and AR1, relative to the whole grain oat flakes used as the initial material, %**

A1 – oat protein concentrate treated with α-amylase; AR1 – protein concentrate treated with α-amylase; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AF1 – oat fibre, treated with α-amylase

3.8. att. Aminoskābju daudzuma procentuālās izmaiņas paraugos A1, AF1 un AR1, attiecībā pret pilngraudu auzu pārslām, kas izmantotas kā kontrole, %

A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi

It is believed that some of the oat protein is bound within the non-starch polysaccharide matrix and could be effectively liberated by enzymes hydrolysing non-starch polysaccharides (Guan & Yao, 2008), in particular glucosidases (McDonald & Tipton, 2021). However, introducing complex enzymes has not changed the amino acid profile in AX1 substantially compared to A1. Interestingly, protein aggregation induced by the shift of ionic strength of the solution has not influenced amino acid redistribution. The amino acid profiles of AXR1 and AX1 only revealed negligible deviations, as shown in Figure 3.9.

Overall, the observed redistribution of amino acids aligns with the previously mentioned profile in which oat flakes were solely treated with α-amylase. A decrease in lysine content was observed in the protein concentrates, whereas a slight increase in lysine was observed in the fibre stream. Additionally, proline was found at lower levels in all samples.

The findings provide clear evidence and demonstrate that the manipulation of ionic strength does not directly initiate the aggregation of different protein fractions. Rather, the increased solution's ionic strength functions as a catalyst for protein aggregation, promoting the attraction and grouping of proteins from the same fraction, or facilitating the formation of aggregates that are inclined to sediment more rapidly. Conversely, the introduction of complex enzymes during the hydrolysis process has a substantial impact on the concentration of amino acids in the fibre stream.

The averaged samples exhibited significantly higher levels of essential amino acids, with the exception of lysine, compared to the recommended values for an ideal protein as outlined in the FAO guidelines (2007). Moreover, the composition of essential amino acids exceeded the recommended values in all the averaged samples. Figure 3.10 illustrates the graph representing the averaged content of essential amino acids in the oat fractions. The averaged samples include A1, AR1, AX1, AR1 as protein fractions, and AF1 and AXF1 as fibre fractions. The summarised content of essential amino acids in averaged samples overcame the recommended 36% ratio for essential/total amino acid content (Jing et al., 2016). Oat protein concentrate demonstrated the highest content of summarised essential amino acids.

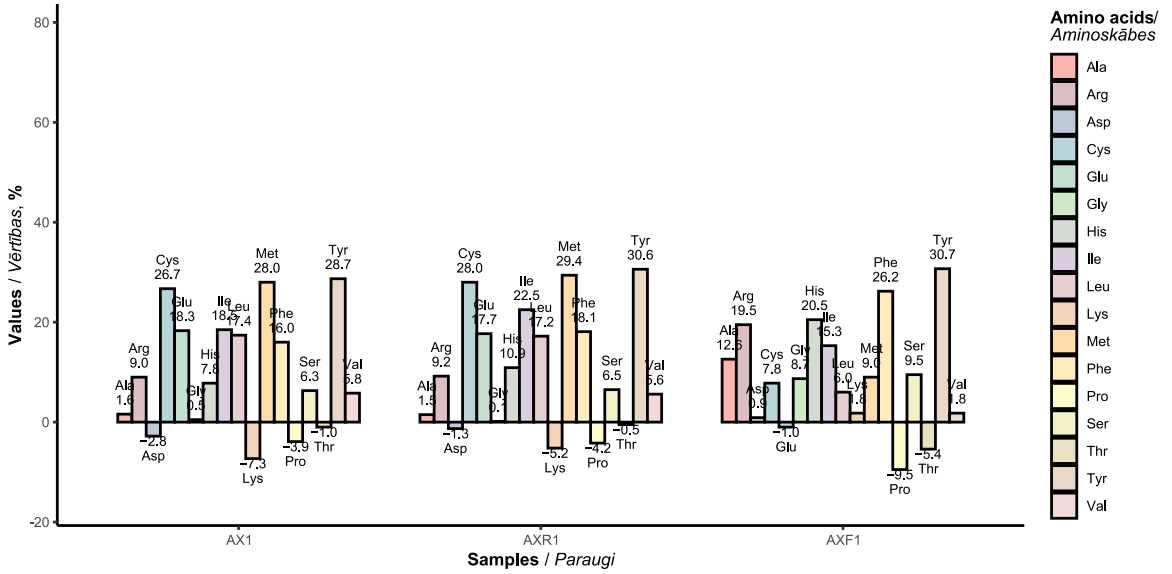


Figure 3.9. **Percentage variation in amino acid quantities observed in samples AX1, AXR1, and AXF1, relative to the initial oat material in the form of whole oat flakes, %**

AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – oat protein concentrate treated with α-amylase, complex enzymes and NaCl; AXF1 – oat fibre, treated with α-amylase and complex enzymes

3.9. att. Aminoskābju daudzuma procentuālās izmaiņas, kas novērotas paraugos AX1, AXR1 un AXF1 attiecībā pret pilngraudu auzu pārslām, kas izmantotas kā kontrole, %

AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – auzu proteīna koncentrāts apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl; AXF1 – auzu šķiedrvielas, apstrādātas ar α-amilāzi un kompleksiem enzīmiem

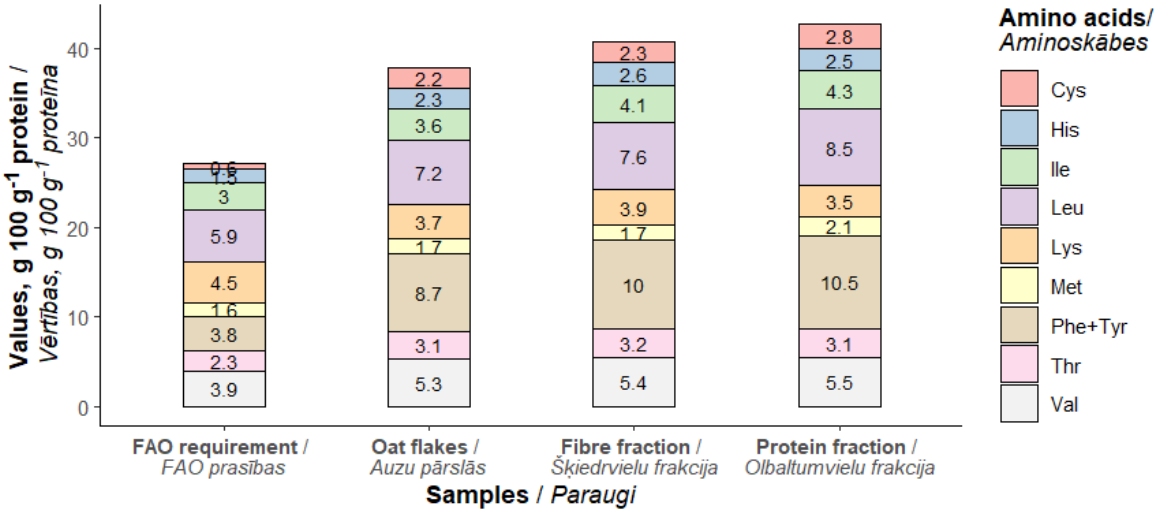


Figure 3.10. **Amount of essential amino acids in initial oat flakes, averaged fibre and protein fractions, and FAO (2007) recommended values, g 100 g^{-1} protein**

Column indicating the FAO requirement reprinted with Ref. permission (FAO et al., 2007)

3.10. att. Neaizvietojamo aminoskābju saturs sākotnējās auzu pārslās, vidējās šķiedrvielu un proteīnu frakcijās un FAO (2007) ieteiktās vērtības, g 100 g^{-1} proteīna

Kolonna, kas norāda FAO prasības, pārpublicēta ar atļauju (FAO, 2007)

Molecular weight

The molecular weight of the protein was determined in two samples, indicated as A1 and AR1. The SDS-PAGE pattern in Figure 3.11 provides the data in reduced samples.

The protein size in both samples displayed variations that fell into two main groups, approximately around 28 kDa and 46 kDa. Sample A1 revealed major bands with 27.8, 45.2 and 51.0 kDa, which comprised 43.3, 28.7 and 24.4% of total protein, respectively. The size of protein in sample AR1 was determined at the level 27.7, 45.3 and 51.0 kDa, which represented 42.5, 28.2 and 24.2% of total protein. The variations among the samples were relatively insignificant; both demonstrated relatively close similarity in terms of size and relative concentration of protein. This confirms that the solution's shifted ionic strength did not impact the quality of agglomerated protein. The aggregated protein displayed uniform protein fractions, while proteins with different sizes, potentially belonging to other protein fractions like albumins, were not detected. In a study by Klose et al. (2009), a prominent band representing oat albumin was reported with a specific molecular weight range of around 35–44 kDa, while other studies have reported a major peak of albumins at 15 kDa (Mirmoghtadaie et al., 2009). However, in the present study, none of these albumin bands were observed. It is possible that the salt-induced protein aggregation led to the aggregation of proteins from the same source. The patterns of the determined bands closely mirror those published earlier for oat globulin size (Klose et al., 2009).

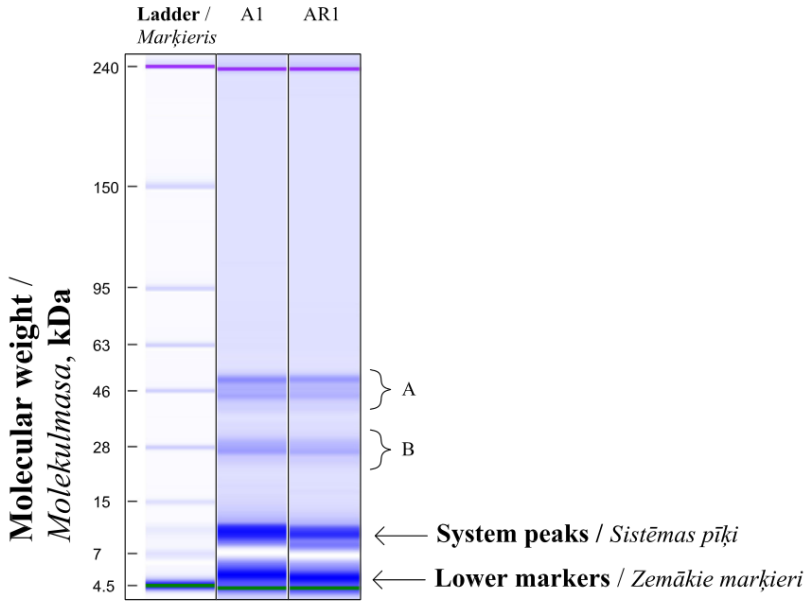


Figure 3.11. **SDS-PAGE image of protein profiles of A1 and AR1 samples, kDa**

Lower markers and system peaks indicated by arrows did not derive from the analysed samples. A1 – oat protein concentrate treated with α-amylase; AR1 – oat protein concentrate treated with α-amylase changing ionic strength

3.11. att. A1 un AR1 proteīna profilu SDS-PAGE attēls, kDa

Apakšējie marķieri un sistēmas pīķi, kas norādīti ar bultiņām, nav iegūti no analizētajiem paraugiem. A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi un iegūt mainot jonu stiprumu

Solubility

The results of the solubility test conducted on samples A1, AR1, and AX1 are illustrated in Figure 3.12. The solubility of the sample A1 was relatively constant across the entire pH

range, with average values of 4.8%, 4.3%, and 4.7% at the pH levels 3, 6, and 9, respectively. Similarly, the solubility of sample AR1 fell within a similar range, averaging at 2.4%, 3.9%, and 5.5% at the investigated pH levels. The protein solubility of the sample AX1 was similarly low at investigated pH levels. It averaged at 4.7%, 4.4% and 6.3% at pH 3, 6 and 9, respectively. It is important to highlight that altering the pH towards either acidic or alkaline conditions only had a minimal impact on protein solubility. This is noteworthy, considering the fact that proteins are typically prone to hydrolysis under harsh pH conditions (Averina, Konnerth, D'Amico, & van Herwijnen, 2021), leading to a reduction in molecular size and subsequently increased solubility. Despite this, the changes observed in protein solubility were not substantial. This observation contrasts with published studies to some extent. Earlier reported solubility of the oat protein, obtained through alkaline solubilisation followed by protein precipitation, ranged from 20% (Walters et al., 2018) to 70% (Zhong et al., 2019). Air-separated native oat proteins were reported as being soluble by more than 20% at pH 7 (Brückner-Gühmann et al., 2018). The solubility of oat proteins which passed enzymatic extraction was reported in the range of 10% to 50% at pH 9 and pH 5, respectively (Prosekov et al., 2018). Protein solubility can be influenced by various factors, ranging from salt concentration (Loponen et al., 2007) to the specific oat variety used in the experiments (Yue, Gu, et al., 2021). It has been reported that purified oat globulins exhibit limited solubility (Loponen et al., 2007). The major bands observed in the current research, falling within the range of 28 to 46 kDa, are likely oat globulins, which could explain the observed limited solubility. The behaviour of oat globulins is significantly affected by salt concentration, particularly in conjunction with extreme pH values.

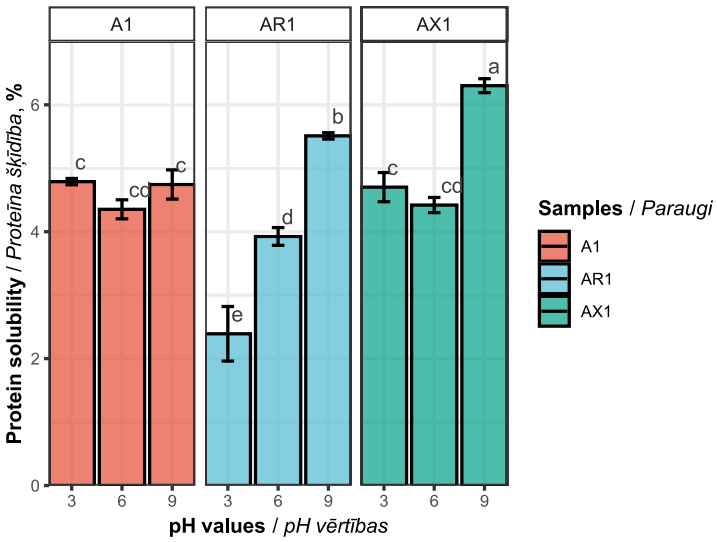


Figure 3.12. **Protein solubility among the oat protein samples A1, AR1, and AX1 at pH 3, 6, and 9; %**

Different letters indicate significant differences within the samples ($p < 0.05$). A1 – oat protein concentrate treated with α-amylase; AR1 – oat protein concentrate treated with α-amylase changing ionic strength; AX1 – oat protein concentrate treated with α-amylase and complex enzymes

3.12. att. Proteīnu šķīdība auzu proteīna paraugiem A1, AR1 un AX1 pie pH 3, 6 un 9; %

Dažādi burti norāda uz būtiskām atšķirībām ($p < 0.05$). A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi, mainot jonu stiprumu; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem

Recent findings indicate that the introduction of NaCl salts can significantly increase solubility, up to 90%, when the NaCl concentration reaches 1 mol L^{-1}. Surprisingly, a low ionic strength solution (around 0.1 *M*) was found to reduce oat protein solubility due to a substantial increase in protein size (R. Li & Xiong, 2021). It is worth mentioning that the methodologies

for protein solubilisation differ, and some limitations have been reported (K. Liu & Hsieh, 2008). The proposed standardised procedure for protein determination in food includes 0.1 *M* NaCl solution (Morr et al., 1985; Sawada et al., 2014), which may potentially reduce protein solubility values, including those observed in the current study.

Foamability

Foaming properties involve two main aspects: foam capacity and foam stability. Foam capacity refers to the overall volume of foam produced under specific conditions, while foam stability is influenced by the rate at which the liquid in the foam precipitates over time (Bairu Zhang, Kang, Cheng, Cui, & Abd El-Aty, 2022).

The foaming capacity of protein concentrate samples was evaluated and expressed as a change in the foam volume and stability within a 2-hour period. Results are presented in Figure 3.13. The protein concentration in the mixture was kept low (1 g of protein per 33 mL of water) to minimise any potential viscosity effects on colloidal stability (Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura, 2017). Samples AXR1 and A1 showed the highest foaming capacity, averaging 7.6% and 7.1% at the initial point, respectively. However, the foam stability was poor across all the samples, with a sharp decline observed over time. AR1 exhibited the lowest foaming capacity and stability, with minimal foam observed at the start of the measurement, averaging at 1.5%, and subsequently disappearing completely in 10 minutes. Interestingly, the variation in ionic strength resulted in diverse foaming characteristics, where AXR1 showed the highest foaming capacity (7.6% at 0 minutes, 4.5% at 120 minutes), while AR1 (1.5% at 0 minutes, 0.0% at 10 minutes) displayed the lowest foaming performance.

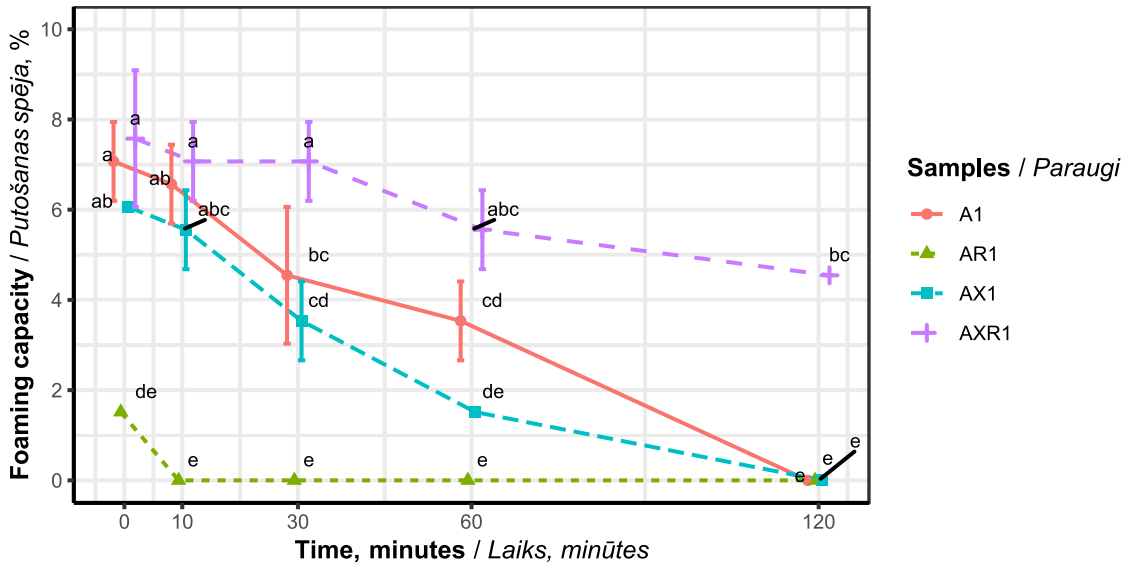


Figure 3.13. **Foaming capacity of oat protein concentrates, %**

Different letters indicate significant differences in each measurement ($p < 0.05$). A1 – oat protein concentrate treated with α-amylase; AR1 – oat protein concentrate treated with α-amylase changing ionic strength; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – oat protein concentrate treated with α-amylase, complex enzymes and NaCl

3.13. att. Auzu proteīna koncentrātu putošanas spēja, %

Dažādi burti norāda uz būtiskām atšķirībām katrā mērījumā ($p < 0.05$). A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi, mainot jonu stiprumu; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl

The foaming capacity observed in our study was notably lower in comparison to the results reported by Kaukonen et al. (2011). In their study, the foaming capacity for oat protein (the protein content in the water extract used for the test was 0.33%, and prior extraction of

lipids was performed using SC-CO_2) reached as high as 137% by volume. However, the water-extracted protein primarily exhibited molecular weights corresponding to bands of 10–15, 20–30, and 35–45 kDa. These molecular weights were relatively smaller than the molecular weight of oat protein. The extraction method used (water extraction) indicates that the reported protein is likely related to the soluble fraction, which may consist of water-soluble albumins. The findings indicate that the foaming capacity of soluble oat proteins is greater than proteins with limited solubility. This aligns with previous reports suggesting that albumins may play a significant role in contributing to foaming *(Runyon, Nilsson, Alftrén, & Bergenståhl, 2013).*

Water/oil holding capacity

The findings regarding the oil and water holding capacities of oat protein concentrates are presented in Figure 3.14. The oil holding capacities of the samples averaged at approximately 2.19 to 1.0. Minimal variation was observed among the samples, with AX1 displaying the highest value and AR1 the lowest, with averaged ratios of 2.21 and 2.16, respectively.

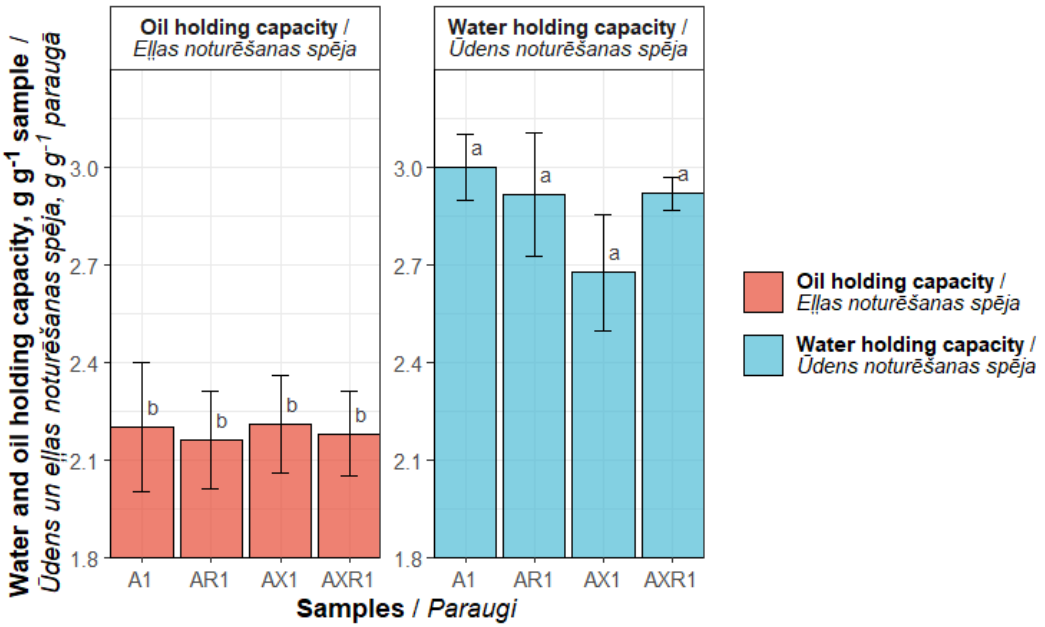


Figure 3.14. **Water and oil holding capacity ratios, g g^{-1}**

Different letters indicate significant differences within the samples ($p < 0.05$). A1 – oat protein concentrate treated with α-amylase; AR1 – protein concentrate treated with α-amylase changing ionic strength; AX1 – oat protein concentrate treated with α-amylase and complex enzymes; AXR1 – protein concentrate treated with α-amylase, complex enzymes and NaCl

3.14. att. Ūdens un eļļas noturēšanas koeficienti, g g^{-1}

Dažādi burti norāda uz būtiskām atšķirībām katrā mērījumā ($p < 0.05$). A1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi; AR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi, mainot jonu stiprumu; AX1 – auzu proteīna koncentrāts, apstrādāts ar α-amilāzi un kompleksiem enzīmiem; AXR1 – proteīna koncentrāts, apstrādāts ar α-amilāzi, kompleksiem enzīmiem un NaCl

The extraction methods showed little impact on the oil holding capacity. Generally, all samples exhibited similar moisture retention when using water. The highest water holding capacity was found in sample A1, with a water-to-protein average ratio of 3.0 to 1.0. On the other hand, the lowest results were observed in samples AR1 and AX1, with ratios averaging 2.84 and 2.83 to 1, respectively. There were no significant differences among the protein samples treated with salts or complex enzymes, with the observed variation being around 5%, which was too low to determine the influence of the presence of salt on the water holding capacity. The oil and water holding capacities observed in this study were higher compared

to the results reported by Mirmoghtadaie et al. (2009). In their study, the water holding capacity was determined to be at a ratio of 1.27 g g^{-1}, while the oil holding capacity was found to be at a ratio of 1.73 g g^{-1} for oat protein obtained through isoelectric precipitation after alkaline extraction. In a similar study, the oat concentrate obtained through alkaline extraction exhibited a water holding capacity ranging from 2.00 to 2.70 mL g^{-1} and an oil holding capacity ranging from 2.25 to 2.80 mL g^{-1} (C. Y. Ma, 1983). Furthermore, the same study also identified that the observed differences in water and oil holding capacities were influenced by the specific oat varieties used in the investigation.

However, in contrast, another study reported that the enzymatically-extracted oat protein concentrate from brans displayed a higher water binding capacity of 3.73 mL g^{-1}, while its oil holding capacity was found to be relatively lower, ranging around 1.26 mL g^{-1} (Prosekov et al., 2018).

3.2.2. Oat protein derived from oat flour / *Auzu proteīns, kas iegūts no auzu miltiem*

Amino acids

Table 3.6 provides the amino acid composition of the analysed protein in the initial raw material, the extracted protein concentrate, and the defatted samples treated with ethanol and supercritical fluid. It also includes the essential amino acid requirements for adults according to FAO recommendations (FAO et al., 2007). The concentration of amino acids expressed per gram of protein in total reflects protein redistribution through the recovery steps.

Tryptophan was not indicated among the measured amino acids due to its decomposition during acid hydrolysis. In comparison to the other amino acid concentrations, the notable abundance of glutamic acid is evident, which is a common occurrence in crops, including oats. The enzymatic hydrolysis of starch resulted in a significant elevation of certain amino acids, namely tyrosine, cysteine, arginine, methionine, isoleucine, with each exceeding a 10% increment. Additionally, a slight increase, less than 10%, was observed for amino acids valine, proline, glutamic acid and aspartic acid. Conversely, a decrease in concentration was determined for lysine, leucine and threonine, accounting for 9%, 9% and 6%, respectively. The decrease in concentration of amino acids such as lysine and alanine might indicate their association with albumins which could have passed into the liquid phase during hydrolysis and separation, given the fact that the concentration of these amino acids in albumins is reported to be higher compared to globulins. The significant elevation in tyrosine concentration may suggest the potential presence of glutelin, as tyrosine is known to be relatively abundant in the oat glutelin fraction (Peterson, 2011).

The use of supercritical CO_2 fluid for lipid removal resulted in a minor decline in the concentration of isoleucine and proline, while the levels of other amino acids showed an increase. Ethanol extraction led to a substantial increase in the concentration of histidine, valine and alanine, which were determined to be higher by 14%, 9%, and 8%, respectively.

The use of supercritical CO_2 fluid for lipid removal resulted in a minor decline in the concentration of isoleucine and proline, while the levels of other amino acids showed an increase. Ethanol extraction led to a substantial increase in the concentration of histidine, valine and alanine, which were determined to be higher by 14%, 9%, and 8%, respectively.

Amino acids have been categorised into two classes based on their polarity: polar and nonpolar. Amino acids classified as nonpolar are free of hydrogen donor or acceptor atoms and these included glycine, phenylalanine, methionine, leucine, isoleucine, proline, alanine and valine. Consequently, side chains are more likely to form clusters and become buried inside the protein matrix. The remaining analysed amino acids were categorised as polar, including both positively and negatively charged amino acids (Vnučec, Kutnar, & Goršek, 2017; Z. Wang et al., 2018; Yue, Zhu, et al., 2021; Zhong et al., 2019). These amino acids are generally considered as hydrophilic and are usually located on the external surface of proteins (Pommie, Levadoux, Sabatier, Lefranc, & Lefranc, 2004). Typically, oat albumins contain a higher

concentration of polar amino acids, while oat globulins show an increased abundance of nonpolar amino acids (Jing et al., 2016).

Table 3.6. / *3.6. tabula*

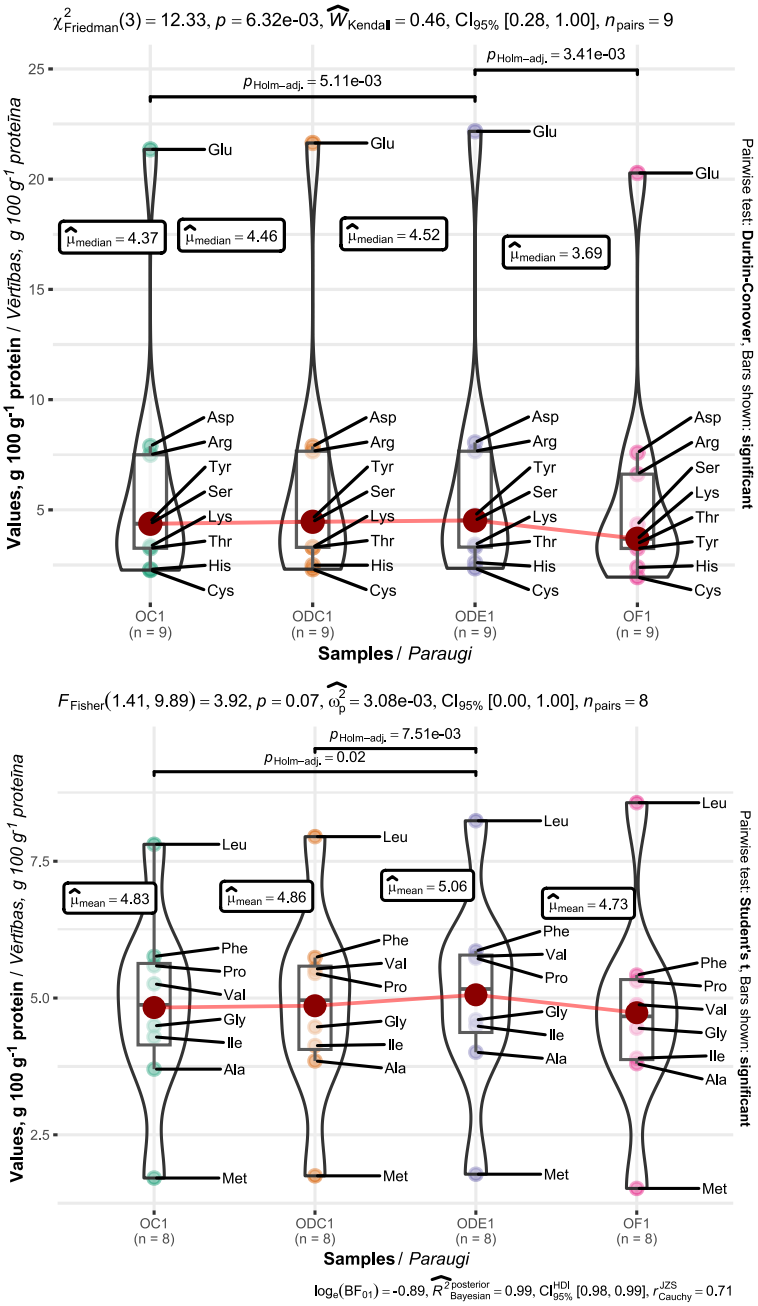
Amino acid composition of samples and essential amino acid requirements in adults by FAO (2007), g 100 g^{-1} of total protein / *Analizēto paraugu aminoskābju sastāvs un neaizstājamo aminoskābju prasības pieaugušajiem pēc FAO (2007), g 100 g^{-1} kopējā proteīna*

Amino acid / *Aminoskābe*	**Oat flour protein** / *Auzu miltu proteīns*	**Experimental samples /** *Eksperimentālie paraugi*			**WHO/FAO, Adults /** *Pieaugušie* **
		OC1	**ODE1**	**ODC1**	
Ala	3.8±0.17a	3.7±0.05a	4.01±0.17a	3.85±0.06a	-
Arg	6.62±0.29b	7.5±0.25a	7.66±0.32a	7.66±0.08a	-
Asp	7.59±0.24a	7.89±0.16a	8.06±0.18a	7.88±0.3a	-
Cys	1.95±0.05b	2.27±0.07a	2.35±0.05a	2.31±0.07a	0.4
Phe	5.42±0.27a	5.76±0.23a	5.86±0.17a	5.74±0.16a	2.5*
Gly	4.45±0.1a	4.49±0.19a	4.6±0.12a	4.47±0.18a	-
Glu	20.28±0.54b	21.35±1.01ab	22.17±0.54a	21.64±0.38ab	-
His	2.39±0.05bc	2.3±0.08c	2.61±0.05a	2.49±0.06ab	1.5
Ile	3.9±0.06b	4.29±0.19ab	4.49±0.15a	4.13±0.19ab	3
Leu	8.57±0.11a	7.81±0.1b	8.24±0.29ab	7.95±0.18b	5.9
Lys	3.69±0.12a	3.34±0.11b	3.42±0.12ab	3.3±0.09b	4.5
Met	1.52±0.08b	1.71±0.02a	1.78±0.04a	1.75±0.08a	1
Pro	5.31±0.23a	5.59±0.18a	5.73±0.17a	5.45±0.06a	-
Ser	4.34±0.18a	4.37±0.1a	4.52±0.15a	4.46±0.16a	-
Tyr	3.25±0.17b	4.47±0.14a	4.75±0.19a	4.61±0.06a	-
Thr	3.47±0.11a	3.26±0.16a	3.31±0.1a	3.3±0.15a	2.3
Val	4.88±0.09c	5.26±0.21bc	5.76±0.14a	5.53±0.14ab	3.90

Data expressed as means ± standard deviations within the row not sharing any letter are significantly different by the ANOVA test at a 5% level of significance. *Phenylanine+tyrosine, ** Reprinted with permission from Ref. (FAO, 2007); OC1 – protein concentrate before defatting; ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol / *Dati, kas izteikti kā vidējie ± standartnovirzes rindā, kuriem nav kopīgi burti, būtiski atšķiras ar ANOVA testu 5% nozīmīguma līmenī. *Fenilanīns+tirozīns, ** Pārpublicēts ar atļauju no Ref. (FAO, 2007); OC1 – proteīnu koncentrāts pirms attaukošanas; ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu*

In terms of polarity, the redistribution of amino acids was minimally affected by the defatting method. While the overall composition of amino acids did not undergo significant changes, the concentration of amino acids increased in both cases, and the aggregated increase was found to be statistically significant for all cases at $p < 0.05$, except for non-polar amino acids extracted using supercritical CO_2 (refer to Figure 3.15). This observation could suggest a reduction in the summarised number of amino acids excluded from the report.

With the exception of lysine, the levels of essential amino acids exceed the requirements set by the FAO for essential amino acids. It is important to note that the recommended values for essential amino acid composition are relative indicators and should be interpreted in context with other influencing factors, such as the efficiency of amino acid utilisation, which significantly depends on the total nitrogen intake in the diet. Higher total nitrogen intake implies lower intake requirements for essential amino acids (FAO et al., 2007).



(a)

(b)

Figure 3.15. **Redistribution of amino acids among the samples: (a) polar amino acids in samples OC1, OF1, ODC1 and ODE1; (b) non-polar amino acids in samples OC1, OF1, ODC1 and ODE1**

Only significant differences within the plot are connected by paths to highlight the significance within the group means or medians. OC1 – protein concentrate before defatting; ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol

3.15. att. Aminoskābju pārdale starp paraugiem: (a) polārās aminoskābes paraugos OC1, OF1, ODC1 un ODE1; b) nepolārās aminoskābes paraugos OC1, OF1, ODC1 un ODE1

OC1 – proteīnu koncentrāts pirms attaukošanas; ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu

Molecular weight of oat protein concentrate isolated from oat fine flour

The molecular weight of oat proteins was investigated to assess whether the enzymatic extraction followed by defatting leads to significant differences in oat protein analysed by SDS-Page in reduced samples. In sample ODC1, the bands with molecular weights of

25.4, 26.8, 42.5, and 48.3 kDa accounted for 16.8, 25.2, 20.4, and 27.7% of the total protein content, respectively. Similarly, in sample ODE1, the bands with molecular weights of 26.3, 27.6, 43.8, and 49.8 kDa represented 23.3, 28.7, 20.8, and 26.0% of the total protein content, respectively. The size dispersion among the samples were evident, with two prominent areas observed at 46 kDa and 28 kDa, labelled as A and B areas in Figure 3.16, respectively. The patterns of both samples displayed similarities, with bands falling within comparable ranges and no significant differences observed.

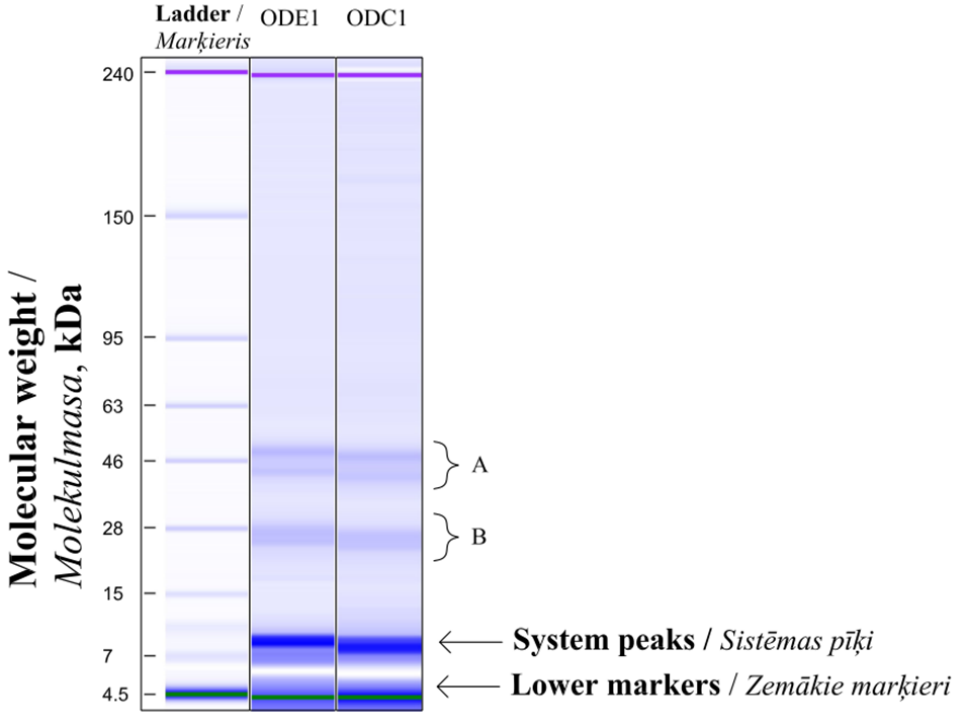


Figure 3.16. **SDS-PAGE image of protein profiles of ODC1 and ODE1 samples**
Lower markers and system peaks indicated by arrows did not derive from the analysed samples; ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol

3.16. att. ODC1 un ODE1 paraugu proteīnu profilu SDS-PAGE attēls
Zemākie marķieri un sistēmas pīķi, kas norādīti ar bultiņām, nav iegūti no analizētajiem paraugiem; ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu

These findings provide evidence of the fact that the size of oat proteins was not affected by the method of lipid extraction. However, some reports have suggested that the electrophoretic profile of oat proteins could be influenced by the defatting treatment itself (Yue, Gu, et al., 2021). In addition, studies have reported that endosperm proteins associated with oil bodies are most prominently observed at the 28 kDa band (Heneen et al., 2008). However, it is important to note that lipids can act as contaminants in electrophoresis, and their removal is assumed to enhance the accuracy of measurements (Wei Wang et al., 2004; Westermeier, 2016). In non-reducing conditions, a 50 kDa band was reported as the main size for oat protein concentrate, while mercaptoethanol-reduced samples showed proteins mostly at 20 kDa and 30 kDa bands, attributed to β and α protein subunits, respectively (Immonen, Chandrakusuma, et al., 2021). The findings from previous studies on fractioned protein are consistent with the results obtained in the current investigation. Specifically, when oat globulins were extracted from oat brans, distinct bands were reported with molecular weights of 15.7, 28.8, 38.8, and 42.7 kDa (Jing et al., 2016). According to the published results, the molecular weight of oat protein investigated in our study closely matches that of the oat globulin fraction (Klose et al., 2009). The similarity in molecular weight can be attributed to the use of similar extraction methods, where a significant portion of protein fractions not associated with globulins was

removed during the extraction process and either remained in the hydrolysate or was bound to the fibre, which was subsequently separated during air separation.

Protein solubility

The solubility of oat protein concentrate samples, namely ODC1 and ODE1, was investigated at various pH levels, and the findings are presented in Figure 3.17. The results showed that the highest solubility was achieved at pH 9, with the ODC1 sample reaching approximately 8.0%. As the pH was lowered to 7 and 5, the solubility decreased to an average value of 6.4% for both measurements. Further lowering the pH to 3 resulted in a slight increase in solubility, reaching around 7.0%. Similar trends were observed for ODE1 samples, with solubility ranging from 6.4% to 9.0% across the pH range from 3 to 9. The differences in solubility among the samples at different pH levels were not significant. In general, the oat protein demonstrated low solubility across the entire pH range tested, and the impact of the lipid extraction method on protein solubility was found to be insignificant. The chosen pH range was predetermined with consideration of its potential use in food-related applications.

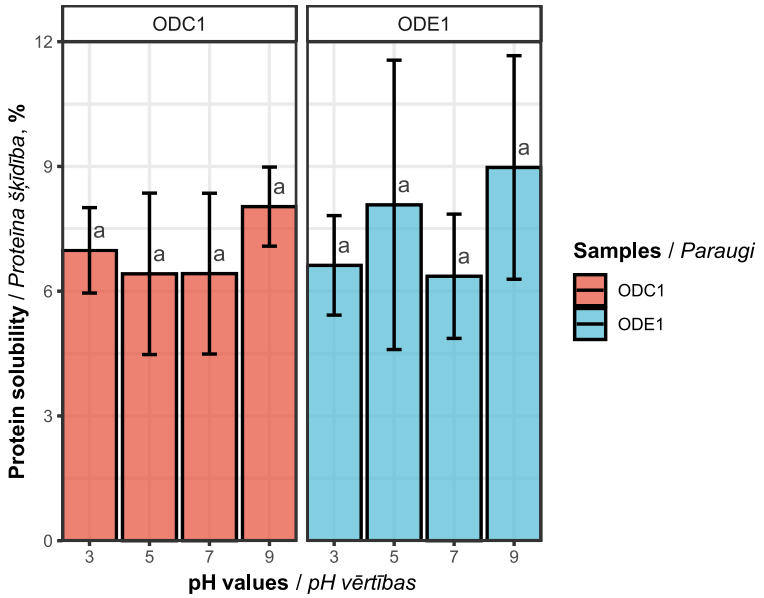


Figure 3.17. **Oat protein solubility in samples ODC1 and ODE1, %**

Means sharing a common letter do not differ significantly at a 5% level of significance. ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol

3.17. att. Auzu proteīna šķīdība paraugos ODC1 un ODE1, %

Vienādi burti parāda nebūtiskas atšķirības 5% nozīmīguma līmenī. ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu

The solubility of oat protein obtained from flour and oat flakes, discussed above, exhibited similar characteristics, with both samples showing relatively low solubility. The pH of the medium had minimal impact on protein solubility, and extreme pH values used in the study did not significantly alter the solubility. For instance, Li & Xiong (2021) reported oat protein solubility reaching up to about 80% at pH 8. However, it is important to note that the reported results were obtained from a specific fraction of oat protein, isolated using NaOH treatment at pH 10, followed by centrifugation, pH readjustment, and drying. Such severe pH treatments might have induced structural changes in the protein, making it more soluble at the same pH levels it was isolated in. It is important to highlight the fact that protein fractions examined in the reported study was extracted at a specific pH and then resuspended, leading most probably to a non-native protein state. Moreover, when isolating a protein fraction at pH 10 and subsequently resuspending it at the same pH, the resulting protein is more likely to

exhibit solubility characteristics similar to that pH level. This observation is a common finding across various studies that have investigated the solubility of proteins following alkaline treatment (Brückner-Gühmann, Benthin, et al., 2019; Mirmoghtadaie et al., 2009; Abdellatif Mohamed et al., 2009; Yue, Gu, et al., 2021). However, the observed low solubility can be primarily attributed to the fact that the isolated protein fraction mainly consisted of a water-insoluble globulin fraction (based on molecular weight bands at about 28 and 46 kDa), which is similar to the protein extracted from oat flakes, as discussed previously.

Water and oil holding capacity

The water and oil holding capacities of defatted oat protein concentrates are illustrated in Figure 3.18. Specifically, sample ODE1 exhibited higher oil and water holding capacities, with average values of 2.18 g g^{-1} and 2.68 g g^{-1}, respectively, in comparison to sample ODC1, which had oil and water holding capacities of 2.05 g $g^{-1}$and 2.61 g g^{-1}, respectively. Measurements across the samples indicated no significant differences, suggesting that the defatting methods, particularly with ethanol or supercritical CO_2, had a minimal impact on the liquid holding capacity. Despite the similar range of results among the extraction methods, there was a substantial difference between the solvents used. Water exhibited a higher binding capacity to the oat protein concentrate than oil in both cases. When compared to protein isolated from oat flakes, the averaged samples displayed slightly lower liquid-holding capacities, approximately 3.7% and 8.3% for oil and water, respectively. This difference may be attributed to variations in the physicochemical composition influenced by the raw material and isolation methods employed. Earlier reports on this matter have shown some inconsistencies, as discussed in previous chapters.

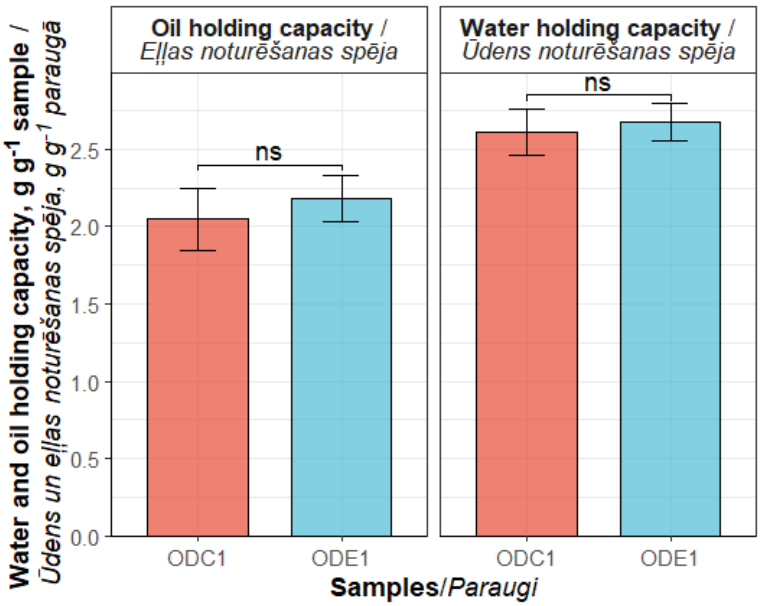


Figure 3.18. **Water and oil holding capacity in samples ODC1 and ODE1, g g^{-1} sample**
Means represented with "ns" are not significantly different by t-test at a 5% level of significance. ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol

3.18. att. ODC1 un ODE1 paraugu ūdens un eļļas noturēšanas spēja, g g^{-1} paraugā
Vidējie, kas attēloti ar "ns", būtiski neatšķiras ar t-testu 5% nozīmīguma līmenī. ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu

Foamability

The foaming properties of the samples are depicted in Figure 3.19. In general, the oat protein concentrate defatted using supercritical fluid CO_2 exhibited higher foamability compared to the sample treated with ethanol. The foaming capacity for sample ODC1 was

initially at 27.3%, remaining stable for 10 minutes, and then gradually decreasing to an average of 21.2% throughout the measurement period. On the other hand, sample ODE1 displayed a lower foaming capacity, starting at 15.2%, sharply declining to 9.1% in 10 minutes, and eventually reaching a final value of 4.5%.

The foaming capacity of the oat protein concentrates in this study was found to be higher than that observed for oat protein extracted from oat flakes; however, it was lower compared to findings reported in the literature. One potential reason for this limited foaming capacity could be the presence of remaining lipids, which may have influenced the observed foaming behaviour. For instance, studies have reported higher foamability in defatted oats treated with hexane, followed by alkaline treatment and isoelectric precipitation. Reported foaming capacity in all samples exceeded 100% and outperformed the foaming capacity of non-defatted oat protein concentrate (Yue, Gu, et al., 2021).

The presence of remaining lipids in the oat protein concentrate might act as a constraining element for its foaming capacity, potentially leading to weaker interactions between proteins adsorbed to lipids or the formation of bridges between protein surfaces (J. Yang, Berton-Carabin, Nikiforidis, van der Linden, & Sagis, 2022). Furthermore, the relatively low solubility of oat protein could be another contributing factor to the limited foaming capacity, as previous studies have highlighted the critical role of protein solubility in determining foaming capacity (Lan, Ohm, Chen, & Rao, 2020; Bairu Zhang et al., 2022). The high insolubility of the protein concentrate observed in the current study further supports this statement.

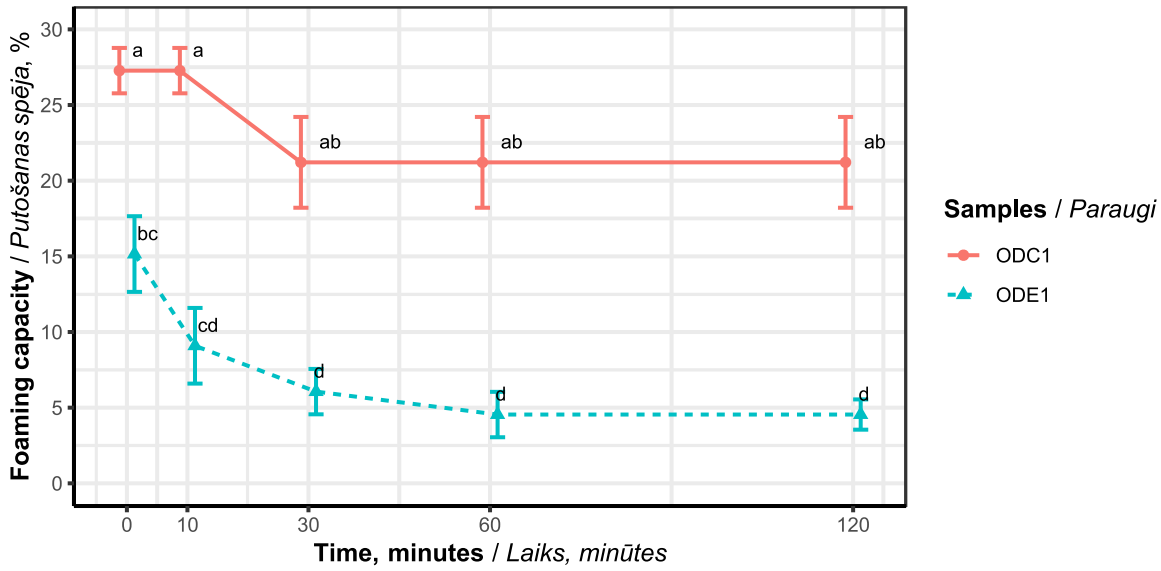


Figure 3.19. **Foaming capacity in samples ODC1 and ODE1, % vol**

Means with no letter in common are significantly different ($p < 0.05$). ODC1 – protein concentrate defatted by SC-CO_2; ODE1 – protein concentrate defatted by ethanol

3.19. att. ODC1 un ODE1 paraugu putošanas spēja, tilp.%

ODC1 – proteīnu koncentrāts, kas attaukots ar SC-CO_2; ODE1 – proteīnu koncentrāts, kas attaukots ar etanolu

Summary of Chapter 3.2 / *3.2 nodaļas kopsavilkums*

The study results suggest that the addition of complex enzymes or adjustments to the ionic strength of the solution during the process of oat protein isolation from oat flakes have a minimal impact on the amino acid profile. Rather, the increased solution's ionic strength functions as a catalyst for protein aggregation, promoting the attraction and grouping of proteins from the same fraction, or facilitating the formation of aggregates that are inclined to sediment more rapidly. Conversely, the introduction of complex enzymes during the hydrolysis process has a substantial impact on the concentration of amino acids in the fibre stream. The amount of

essential amino acids in the extracted oat protein exceeded the recommendations outlined by FAO, except for lysine (FAO et al., 2007). Additionally, the defatting method had a negligible influence on the nutritional value of the protein in terms of its amino acid composition. The molecular analysis of oat protein revealed major bands at approximately 28 and 46 kDa, corresponding to oat globulin. However, the protein exhibited a relatively low performance in terms of solubility and foaming capacity, irrespective of the extraction method and initial raw material used. The liquid retention for oil and water fell within the range of approximately 2.05–2.21 g g^{-1} and 2.61–3.00 g g^{-1}, respectively.

Pētījuma rezultāti liecina, ka komplekso fermentu pievienošana vai šķīduma jonu stipruma korekcijas, izolējot auzu proteīnu no auzu pārslām, minimāli ietekmē aminoskābju profilu. Drīzāk palielināts šķīduma jonu stiprums darbojas kā proteīna agregācijas katalizators, veicinot vienas un tās pašas frakcijas olbaltumvielu piesaisti un grupēšanos vai sekmējot tādu agregātu veidošanos, kas ātrāk nogulsnējas. Turpretī komplekso fermentu izmantošana hidrolīzes laikā būtiski ietekmē aminoskābju koncentrāciju šķiedrvielu frakcijā. Neaizvietojamo aminoskābju daudzums saturs auzu proteīnā pārsniedza Pārtikas un lauksaimniecības organizācijas (FAO) ieteikumos norādīto līmeni, izņemot lizīnu (FAO, 2007). Papildus tam, attaukošanas metodes ietekme uz proteīna uzturvērtību aminoskābju sastāva ziņā bija nenozīmīga. Auzu proteīna molekulārā analīze uzrādīja galvenās joslas aptuveni 28 un 46 kDa, kas atbilst auzu globulīnam. Tomēr proteīns uzrādīja salīdzinoši zemu šķīdību un putošanas spēju, neatkarīgi no ekstrakcijas metodes un izmantotās sākotnējās izejvielas. Paraugu eļļas un ūdens noturēšanas spēja bija attiecīgi aptuveni 2,05–2,21 g g^{-1} un 2,61–3,00 g g^{-1}.

3.3. Extrusion of oat protein concentrate / *Auzu proteīna koncentrāta ekstrūzija*

The primary aim of current study is to assess the suitability of the oat protein obtained for implementation in wet extrusion systems utilising a single screw extruder. Subsequently, the extrudate is subjected to analysis to evaluate its texture profile, colour, and microstructure. To conduct the extrusion process, a sample of oat protein concentrate defatted using ethanol (referred to as ODE1) was employed, alongside a commercial soy protein concentrate as a reference material. Among the various tested samples, ODE1 exhibited the most promising potential for extrusion, primarily due to its fibre content meeting industry standards and the practicality and cost-effectiveness of the ethanol defatting method, as previously discussed.

3.3.1. Extrusion / *Ekstrūzija*

The extrudate, as observed empirically, can be described as a firm, well-formed solid with a dark pale brown colour and rare visible cracks. During the extrusion process, vapour was noticeable, likely due to the absence of cooling at the die, causing the extruded material to be released in a free form at the temperature resulting from decompression. The extrudate did not exhibit a tendency to expand, maintaining dimensions close to those of the slit, and breaking into non-regular length pieces. Cooled to room temperature, the extrudate became difficult to slice. Figure 3.20 illustrates cross-sectional and longitudinal views of oat protein extrudate samples.

It has been mentioned that achieving a protein content within the range of 50% to 70% is essential to facilitate the formation of fibrous structures during extrusion (Immonen, Chandrakusuma, et al., 2021). However, several other key factors may exert an influence on the formation of fibrous structures in oat proteinaceous materials, including pressure, cooking time, temperature, and the inclusion rate of water or other crop compounds such as starch (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014; Sargautis, 2020). Recent reports have highlighted the significance of determinants such as temperature and moisture in shaping the

fibrous structure. Notably, when one of the mentioned parameters is kept constant while increasing the other, the formation of fibrous structures is negatively affected (J. Zhang et al., 2020). The role of shear force and its direction in fibrous structure formation has also been emphasised. It is conceivable that specific determinants, such as moisture content, may influence shear force, serving as an indicator of a material's capability to form fibrous structures. Additionally, the oil content in the initial material emerges as another noticeable factor. As a substantial portion of oil typically remains on the protein side during protein concentration, its high content hinders or even precludes the proper formation of extrudates. Reduced friction in the extrusion system constitutes one of the factors hindering the appropriate formation of extrudates. Experiments conducted by the author revealed that an oil content of 20% diminishes the extrudate's ability to form a solid structure, resulting in a loose form that is susceptible to breaking down, lacking a visible molten protein matrix. Moreover, setting the pressure with the same die mentioned at a constant level becomes unfeasible due to the lack or reduction of shear forces, leading to a dynamic state.

Figure 3.20. **Samples of oat protein extrudate, cross and longitudinal section /**
3.20. att. Auzu proteīna ekstrudāta paraugi, šķērsgriezums un garengriezums

It is posited that the high protein and dietary fibre content play a role in reducing extrudate expansion (Beck et al., 2018). Moreover, high protein content initiates clustering, porosity, and cell thickness within the extrudate. During the high moisture extrusion process, the formation of protein aggregation increases noticeably, resulting in reduced solubility and foaming capacity of the extrudates due to severe effects on disulphide bonds that influence structure formation (Bairu Zhang et al., 2022). However, an increase in moisture content leads to a decrease in free sulfhydryl levels in the extrudates. Chanvrier et al. (2005) observed the lack of homogeneity and protein reorganisations into larger-size aggregates during corn protein extrusion. It is worth mentioning that protein reorganisation is typically discussed in the presence of starch, thus the observations of the lack of homogeneity of the sample should be seen in this context. Recent studies have speculated that the involvement of protein in the formation of the anisotropic structure of the extrudate may be limited, indicating a negligible extent of covalent protein bonding (Wittek, Zeiler, Karbstein, & Emin, 2021). In particular, Wittek et al. (2021) proposed that the anisotropic structure in soy protein isolate during extrusion is primarily related to the formation of insoluble protein clusters around the dispersed water phase, which may involve water-soluble proteins and other polymers interacting in the extrusion system. They suggested that insoluble aggregated proteins with low solubility and high molecular weight were bonded through isopeptide bonds during the pre-processing of soy protein isolate.

The process of extrudate formation was observed to initiate within the A-B sections indicated by arrows in Figure 3.21. Protein melting was observed during the last 2–3 pitches of the screw, with previous pitches functioning solely as transportation and precooking systems. The material inside the barrel before the A-B area appeared unfirm, bright, and without noticeable signs of melting formation.

For validation purposes, a soy protein concentrate was selected as a reference material for extrusion, aiming to compare and assess the chosen extrusion procedures. This selection was based on the similarity in material composition and structure between soy protein and oat protein. Furthermore, soy protein concentrates have commonly been favoured as suitable choices in extrusion systems that utilise a single source of protein (Thadavathi, Wassén, & Kádár, 2019).

Figure 3.21. **Extrudate formation sections**
Arrows A and B point to the region where the initiation of extrudate formation was observed
3.21. att. Ekstrudāta veidošanas sekcijas
Bultiņas A un B norāda uz reģionu, kurā tika novērota ekstrudāta veidošanās sākšanās

3.3.2. Texture and colour of extrudates / *Ekstrudātu struktūra un krāsa*

Texture

The results of texture profile analysis of the extrudates are illustrated in Figure 3.22. Notably, the hardness of oat protein extrudate, with a mean value of 176.9 N exceeded that of soy protein concentrate, which exhibited an average hardness of 143.4 N. While the hardness measurements for oat protein were not uniform within the oat sample, the spread of measurements for the referenced soy protein extrudate displayed a similar level of variability, leading to the determination that the disparity between the two samples was statistically insignificant. The observed differences in hardness measurements could potentially be attributed to variations in the composition of the samples, thereby exerting an impact on the overall texture of the extrudates (Lobato, Anibal, Lazaretti, & Grossmann, 2011). It is essential to acknowledge that the hardness of extrudates is influenced by their protein content, with higher protein levels generally leading to reduced hardness (Sun, Sun, Jia, Sun, & Cao, 2011). Similarly, the oil content also plays a role in determining the hardness of the extrudates; the elevated oil content contributed to lower hardness in certain measurements (H. Wang et al., 2023). The findings suggest that the oat extrudates achieved a peak hardness of 205.0 N in some measurements. Nevertheless, it is essential to consider customer preferences, particularly for certain product categories such as snacks, where the acceptable hardness level is believed not to surpass 200 N.

Fracturability, which reflects the brittleness or crunchiness of products, is commonly assessed through peak force measurements (Linly et al., 2021). In this study, the fracturability of oat protein extrudate exhibited a wide range, spanning from 87.0 to 205.0 N, with a calculated mean of 148.0 N. In contrast, the fracturability of soy protein extrudate demonstrated a narrower range, ranging from 88.7 to 122.4 N, and a mean value of 103.7 N. The noticeable variance in fracturability observed in the oat protein extrudate appears to be linked to its lower homogeneity. Despite its seemingly relatively uniform plain surface, fractures were evident at cross sections, contributing to the observed variability. In comparison, the surface of soy protein extrudate displayed visible roughness in contrast to oat protein extrudate. Despite the noticeable difference in the fracturability mean values between the two analysed samples, the notably high variability in measurements prevented the conclusive establishment of statistically significant differences.

Toughness, representing the total positive area under the curve and quantifying the total work exerted during the test, exhibited higher values in oat protein extrudates, with an average

of 348.6 N × s. In comparison, soy extrudate displayed an average toughness value of 292.0 N × s. However, despite the observed difference in average toughness values between the samples, statistical analysis indicated that the means of toughness were not significantly different.

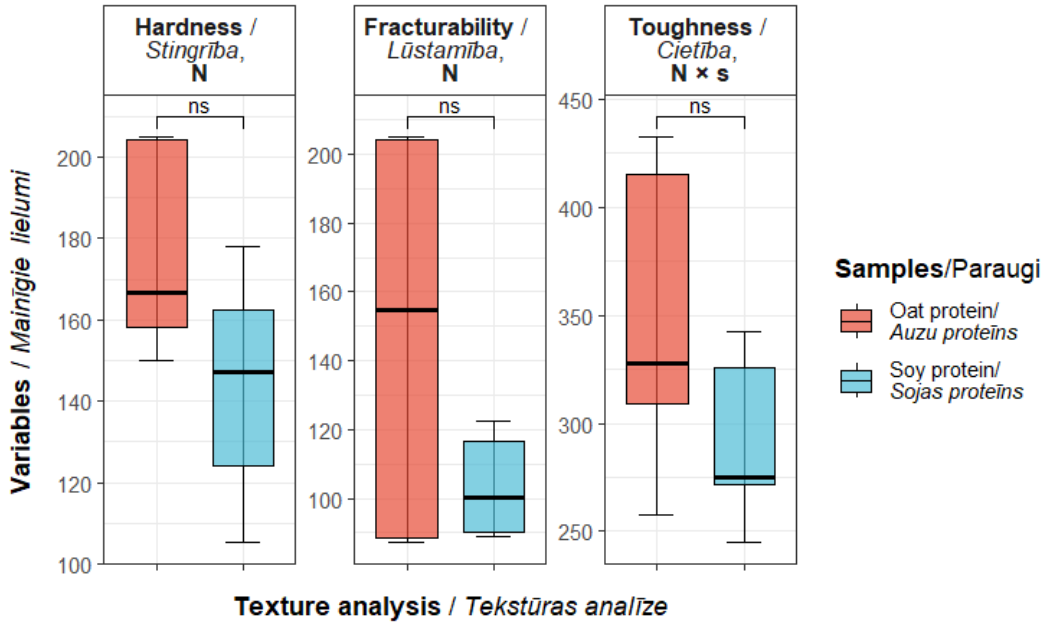


Figure 3.22. **Texture profile of oat and soy protein extrudates, displaying toughness, fracturability and hardness in samples**

Means represented with "ns" are not significantly different by t-test at a 5% level of significance

3.22. att. Auzu un sojas proteīna ekstrudātu struktūra, kas parāda paraugu stingrību, lūstamību un cietību

Vidējie, kas attēloti ar "ns", būtiski neatšķiras ar t-testu 5% nozīmīguma līmenī

Colour

The colour attributes of oat and soy extrudates were documented in Table 3.7. Evaluated as tri-stimulus attributes, the colour of the oat extrudate appeared darker, with an average value of 21.78, compared to soy extrudates whose mean was 48.68, despite the initial colour similarity of the raw materials. A darker colour is generally associated with higher protein content, as the L value exhibits a negative correlation with protein content (Agrahar-Murugkar, Gulati, Kotwaliwale, & Gupta, 2015). Recent research has indicated the influence of oil content on lightness during the extrusion of soy protein isolate, whereby an increase in oil concentration from 0 to 8% resulted in a shift of L values from 41.7 to 53.8 (H. Wang et al., 2023).

Table 3.7. / *3.7. tabula*

CIE lab colour parameters of oat and soy raw materials and extrudates /
Auzu un sojas izejvielu un ekstrudātu CIE krāsu parametri

Sample / *Paraugs*	**a***	**b***	**L***
Oat raw material / *Auzu izejviela*	2.60±1.41 a	11.57±5.36 a	83.6±2.47 b
Oat extrudate / *Auzu ekstrudāts*	0.61±1.56 ab	11.34±5.68 a	21.78±5.27 d
Soy raw material / *Sojas izejviela*	−0.67±2.38 b	14.19±5.61 a	90.68±2.49 a
Soy extrudate / *Sojas ekstrudāts*	−0.54±1.55 b	15.48±4.73 a	48.68±3.44 c

Means ± standard deviations within the column not sharing any letter are significant different by ANOVA test at a 5% level of significance / *Vidējie ± standartnovirzes kolonnā, kuriem nav vienādi burti, būtiski atšķiras ar ANOVA testu 5% nozīmīguma līmenī*

However, in the present study, no significant colour shift was observed in the a* direction, indicating that the colour of the extruded samples did not significantly differ from that of the raw materials. Previous literature has suggested that the development of redness in extruded products might be associated with Maillard reactions inducing browning (Kristiawan et al., 2018). Nevertheless, in the present study the changes towards both the red and blue directions were relatively small and insignificant. The colour changes between the raw material and processed material expressed as ΔE averaged at 42.57±5.63 and 62.14±4.56 for soy and oat protein extrudates, respectively. Such perceivable colour alterations were relatively high and could be analytically described as substantial (Andrés, Villanueva, & Tenorio, 2016).

3.3.3. Microstructure of the extrudate / *Ekstrudāta mikrostruktūra*

In this research, a scanning electron microscope was utilised to investigate the structure of oat protein concentrate. The purpose was to focus solely on the extrusion of the inherent components of the protein concentrate and avoid any potential interference from major grain components, such as starch or other admixed substances, which are considered to have an impact on the extrusion process and attributes characterising extrudates. The images displaying protein microstructure at different magnifications, with scale bars representing 20 μm and 200 μm, are presented in Figure 3.23. The magnification levels and power of 15 kV were chosen empirically as best representing the structure of extruded oat protein. At closer magnification the images reveal the protein surface at a cellular level, while the higher magnification provides an overview of the overall structure, demonstrating its homogeneity and orientation.

Specifically, Figure 3.23a represents the initial formation of the protein extrudate at Point A in Figure 3.21. This structure appeared loose, ruptured, and did not exhibit the tendency of melting. Subsequently, in Figure 3.23d, the picture illustrates the onset of protein concentrate melting before the exit of the extruder (Point B in Figure 3.21). Although the sample showed partial melting, no solid structure was visible. Ruptures and aggregation into anisotropic formations were observed, and the intermediate structure tended to break down easily into smaller formations.

In contrast, tangential sections of the extruded product in Figures 3.23c and 3.23f revealed a solid extrudate that was relatively hard to cut and had changed in colour to pale brown. Further examination through the longitudinal slice showed that the material's texture exhibited relative orientation towards the direction of extrusion. The surface exhibited smoothness and molten characteristics, but no evidence of forming a fibrillar structure was observed. The internal breaks within the structure were potentially formed due to water evaporation, possibly caused by insufficient cooling during the extrusion process. The release of air during extrusion may serve as a precondition for the development of the ruptured structure. The presence of noticeable aggregates and clusters, as seen in Figure 3.23c, could be attributed to the collapse of air cells. Previous studies have indicated that the collapse of air bubbles during extrusion can lead to a crater-like structure formation, particularly when the protein content in the extrudate is high (Beck et al., 2018). The oat protein concentrate under investigation contained a portion of unextracted fibre, which became concentrated alongside protein during processing. The increased fibre content led to a corresponding increase in cell density, where air bubbles were formed. Since brans are mainly composed of insoluble fibre and undergo limited changes in solubility during extrusion (Robin, Dubois, Pineau, Schuchmann, & Palzer, 2011), they act as nucleation agents for bubble formation due to their inability to be wetted. Additionally, the increase in bran content reduced cross-sectional volumetric expansion, although this effect was found to be insignificant in the investigated sample. Previous research has indicated an increase in longitudinal expansion with increased bran content (Robin et al., 2011), although this specific parameter was not measured in the current study. The cross-section of the extrudate, as depicted

in Figure 3.23(b, e), provided a similar view, with the structure orientation of the extrudate described as anisotropic.

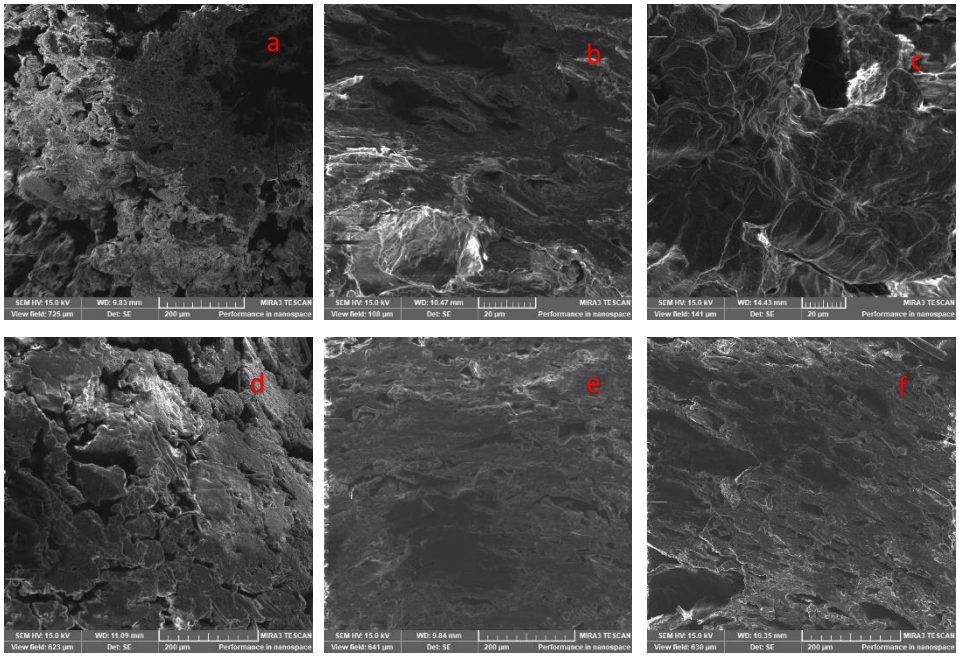


Figure 3.23. **Microstructure of oat protein extrudate at different stages of extrudate formation and different magnification**

(a) beginning of the extrusion, longitudinal view, scale bar – 200 µm; (b) cross-section scale, bar – 20 µm, (e) cross-section, scale bar – 200 µm; (d) intermediate, scale bar – 200 µm; (c) longitudinal view, scale bar – 20 µm, and (f) longitudinal view, scale bar – 200 µm. Images that are not labelled as "beginning" or "intermediate" stages refer to the extruded oat protein concentrate

3.23. att. Auzu proteīna ekstrudāta mikrostruktūra dažādos ekstrudāta veidošanās posmos un dažādos palielinājumos

(a) ekstrūzijas sākums, garenskats, mēroga josla – 200 µm; b) šķērsgriezums, mēroga josla – 20 µm, e) šķērsgriezums, mēroga josla – 200 µm; d) starpposms, mēroga josla – 200 µm; (c) garenskats, mēroga josla – 20 µm un (f) garenskats, mēroga josla – 200 µm. Attēli, kas nav apzīmēti kā "sākuma" vai "starpposma" posmi, attiecas uz ekstrudētu auzu proteīna koncentrātu

Summary of Chapter 3.3 / *3.3 nodaļas kopsavilkums*

Extrusion of defatted oat protein concentrate resulted in the formation of a solid product characterised by a firm, well-formed, and dark pale brown colour. Surface cracks were infrequently observed. The process of protein melting was noticed in the last 2–3 pitches of the single screw extruder; however, the development of a fibrous structure was not evident. The textural properties of the oat extrudates, including hardness, fracturability and toughness, surpassed those of the referenced soy protein extrudate. Nevertheless, these differences did not reach statistical significance due to the relatively wide spread of observations caused by the non-uniform structure of the oat protein extrudate.

The colour values of the oat protein extrudate were darker compared to the referenced soy protein. However, when assessing the colour change using the CIE L*a*b colour system, the shift towards red and blue directions from the initial material was insignificant.

Microstructural analysis of the oat material revealed that extrudate formation initiated in the last 2–3 pitches of the screw, resulting in a loose, ruptured structure without a noticeable tendency for melting. Examination of the intermediate structure before exiting the extruder indicated partial melting and the development of anisotropic formations. In the subsequent final stage of extrusion, the extrudate structure displayed relative orientation towards the direction

of extrusion. Observed breaks inside the extrudate may potentially be attributed to water evaporation. The presence of noticeable aggregates and clusters in the extrudate is assumed to have resulted from the collapsing of air bubbles.

Ekstrudējot attaukotu auzu proteīna koncentrātu, izveidojās cietviela ar stingru, labi izveidotu formu un bija tumšā smilškrāsā. Virsmas plaisas tika novērotas reti. Proteīna kušana tika novērota vienskrūves ekstrūdera pēdējos 2–3 vītnes soļos, taču šķiedrainas struktūras veidošanās netika novērota. Auzu ekstrudātu tekstūras īpašības, tostarp cietība, trauslums un stigrība, pārspēja kontroles sojas proteīna ekstrudāta īpašības. Tomēr šīs atšķirības nebija statistiski nozīmīgas, jo novērojumi bija samērā plaši sadalīti auzu proteīna ekstrudāta neviendabīgās struktūras dēļ.

*Auzu proteīna ekstrudāta krāsas vērtības bija tumšākas salīdzinājumā ar kontroles sojas proteīnu. Tomēr, novērtējot krāsu izmaiņas, izmantojot CIE L*a*b krāsu sistēmu, nobīde sarkanā un zilā virzienā no sākotnējā materiāla bija nenozīmīga.*

Auzu materiāla mikrostruktūras analīze uzrādīja, ka ekstrudāta veidošanās sākās pēdējos 2–3 skrūves vītnes soļos, kā rezultātā izveidojās irdena, pārrauta struktūra bez ievērojamas kušanas tendences. Pārbaudot starpstruktūru pirms izvadīšanas no ekstrūdera, tika konstatēta daļēja kušana un anizotropu veidojumu attīstība. Turpmākajā pēdējā ekstrūzijas posmā ekstrudāta struktūra bija relatīvi orientēta ekstrūzijas virzienā. Novērotie pārrāvumi ekstrudāta iekšpusē, iespējams, ir saistīti ar ūdens iztvaikošanu. Tiek pieņemts, ka ievērojami agregāti un klasteri ekstrudātā ir radušies gaisa burbuļu plīšanas rezultātā.

CONCLUSIONS / *SECINĀJUMI*

1. The wet enzymatic extraction method proves to be effective in recovering oat protein, yielding concentrations of up to 85% for protein isolated from whole oat flakes with suspended fibre solids removed and 78% for protein extracted from oat flour with reduced initial fibre content.
2. The addition of ionisable salts, specifically NaCl at a concentration of 0.1 M, leads to a significant enhancement in oat protein recovery, increasing it by up to 24.8%.
3. The introduction of complex enzymes or alterations to the ionic strength of the solution during the procedure of oat protein isolation from whole oat flakes has a limited influence on the amino acid profile of the resulting protein.
4. Ethanol treatment and supercritical CO_2 treatment significantly reduce the lipid content in oat protein concentrates ODE1 and ODC1 from 20.6 % to 4.9% and 3.5% respectively, while demonstrating minimal effects on the protein's nutritional value, as proven by negligible changes in its amino acid composition.
5. The amount of essential amino acids in the extracted oat protein exceeded the recommendations for adults outlined by FAO, except for lysine.
6. Oat protein concentrates passed through enzymatic extraction comprise a predominantly oat globulin fraction, as evidenced by SDS-page patterns showing dominated protein bands at approximately 28 and 46 kDa.
7. The obtained oat protein exhibited relatively low performance in terms of solubility and foaming capacity, irrespective of the extraction method and initial raw material used. The liquid retention for oil fell within the range of 2.05 to 2.21 g g^{-1}, while for water, it ranged from 2.61 to 3.0 g g^{-1}.
8. Utilising a single screw extruder for the extrusion of pure oat protein concentrate yields a dense, dark pale brown product with a firm texture that surpasses soy protein extrudate in terms of hardness, fracturability, and toughness.
9. The thesis revealed that the use of pure oat protein concentrate in wet extrusion processes is feasible; however, optimising its textural properties may require the incorporation of additional ingredients tailored to the specific application objectives.

1. *Mitrās fermentatīvās ekstrakcijas metode ir efektīva auzu proteīna atgūšanā, sasniedzot koncentrācijas līdz 85% proteīnam, kas izolēts no pilngraudu auzu pārslām, no kurām atdalītas suspendētās cietās šķiedrvielas, un 78% proteīnam, kas ekstrahēts no auzu miltiem ar samazinātu sākotnējo šķiedrvielu saturu.*
2. *Pievienojot jonizējamus sāļus, konkrēti NaCl 0,1 M koncentrācijā, ievērojami uzlabojas auzu proteīna atgūstamība, palielinoties līdz pat 24,8%.*
3. *Komplekso fermentu izmantošanai vai šķīduma jonu stipruma izmaiņām auzu proteīna izolēšanas procesā no pilngraudu auzu pārslām ir ierobežota ietekme uz iegūtā proteīna aminoskābju profilu.*
4. *Apstrāde ar etanolu un apstrāde ar superkritisko CO_2 nozīmīgi samazina tauku saturu auzu proteīna koncentrātos ODE1 un ODC1 no 20,6% līdz attiecīgi 4,9% un 3,5%, vienlaikus minimāli ietekmējot proteīna uzturvērtību, par ko liecina nenozīmīgas izmaiņas tā aminoskābju sastāvā.*
5. *Neaizvietojamo aminoskābju saturs ekstrahētajā auzu proteīnā pārsniedza FAO ieteikumos norādīto līmeni pieaugušajiem, izņemot lizīnu.*
6. *Auzu proteīna koncentrāti, kas ekstrahēti ar fermentatīvo ekstrakciju, pārsvarā sastāv no auzu globulīna frakcijas, par ko liecina SDS-PAGE analīzes rezultāti, kuros dominē proteīna joslas aptuveni 28 un 46 kDa.*
7. *Iegūtais proteīns uzrādīja salīdzinoši zemu šķīdību un putošanas spēju, neatkarīgi no ekstrakcijas metodes un izmantotās sākotnējās izejvielas. Eļļas noturēšanas spēja bija robežās no 2,05 līdz 2,21 g g^{-1}, bet ūdens noturēšanas spēja–no 2,61 līdz 3,00 g g^{-1}.*

8. *Izmantojot vienskrūves ekstrūderi tīra auzu proteīna koncentrāta ekstrudēšanai, iegūst blīvu produktu tumšā smilškrāsā ar stingru konsistenci, kas pēc cietības, trausluma un stigrības pārspēj sojas proteīna ekstrudātu.*
9. *Promocijas darbā tika noskaidrots, ka tīra auzu proteīna koncentrāta izmantošana mitrās ekstrūzijas procesos ir iespējama, tomēr, lai optimizētu tā tekstūras īpašības, var būt nepieciešams iekļaut papildu sastāvdaļas, kas pielāgotas konkrētiem izmantošanas mērķiem.*

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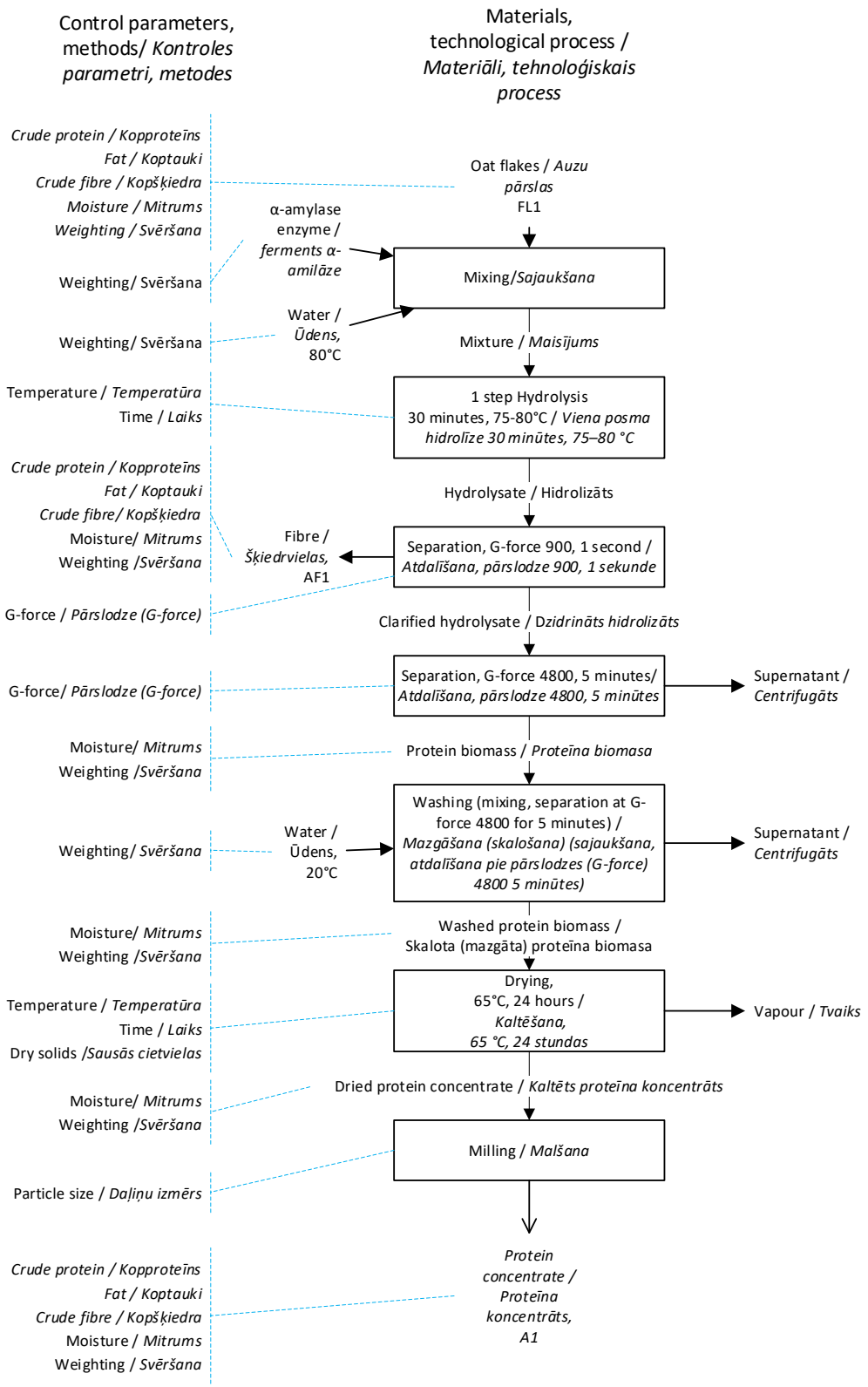
APPENDIXES / *PIELIKUMI*

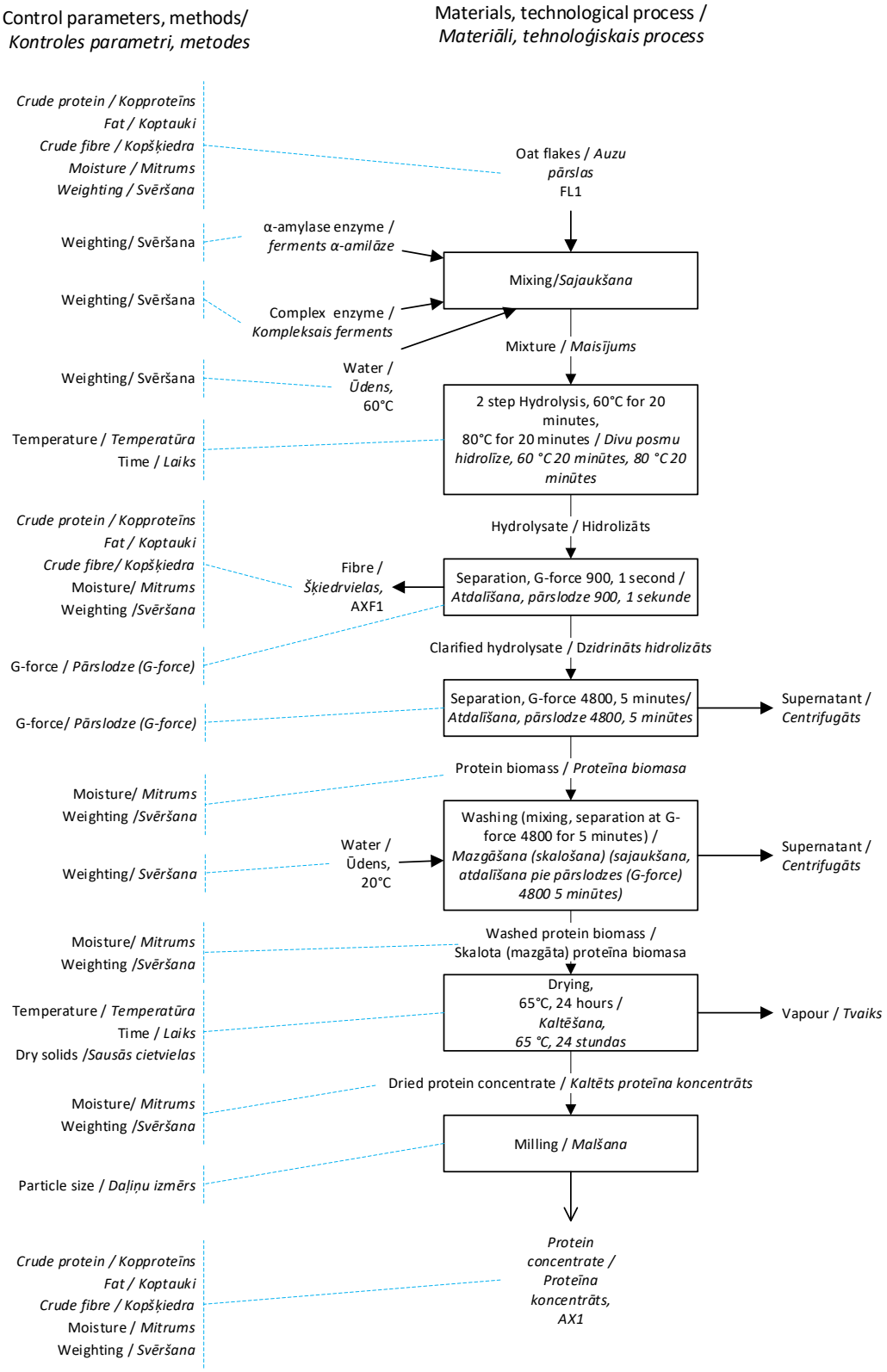


Scheme of oat protein extraction hydrolysing starch by α-amylase

Control parameters and methods describe measurements and applied analysis used for the particular process

Auzu proteīna ekstrakcijas shēma, hidrolizējot cieti ar a-amilāzi

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

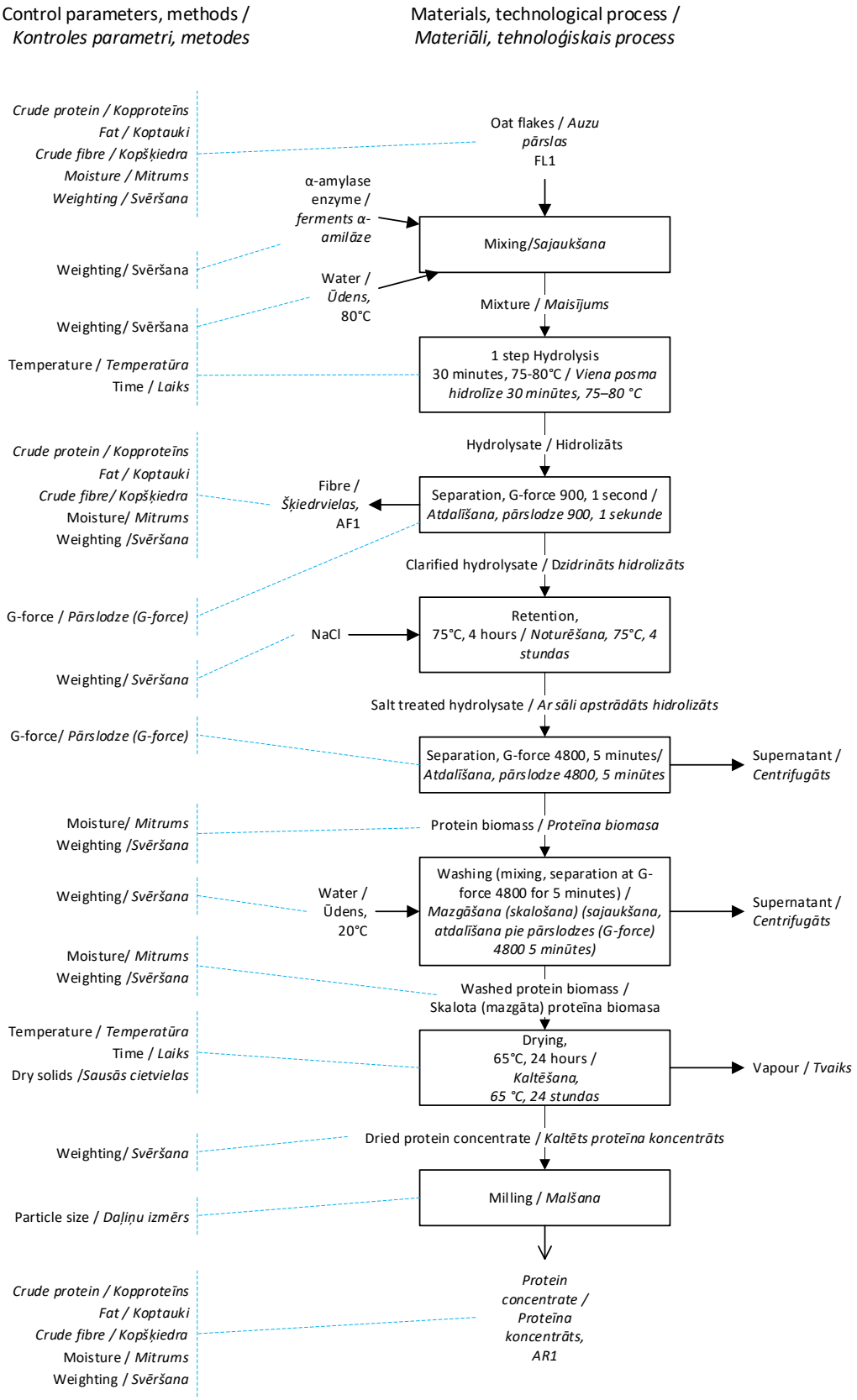


Scheme of oat protein extraction hydrolysing starch by α-amylase and complex enzymes for non-starch polysaccharides

Control parameters and methods describe measurements and applied analysis used for the particular process.

Auzu proteīna ekstrakcijas shēma, hidrolizējot cieti ar a-amilāzi un kompleksajiem fermentiem cieti nesaturošiem polisaharīdiem

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

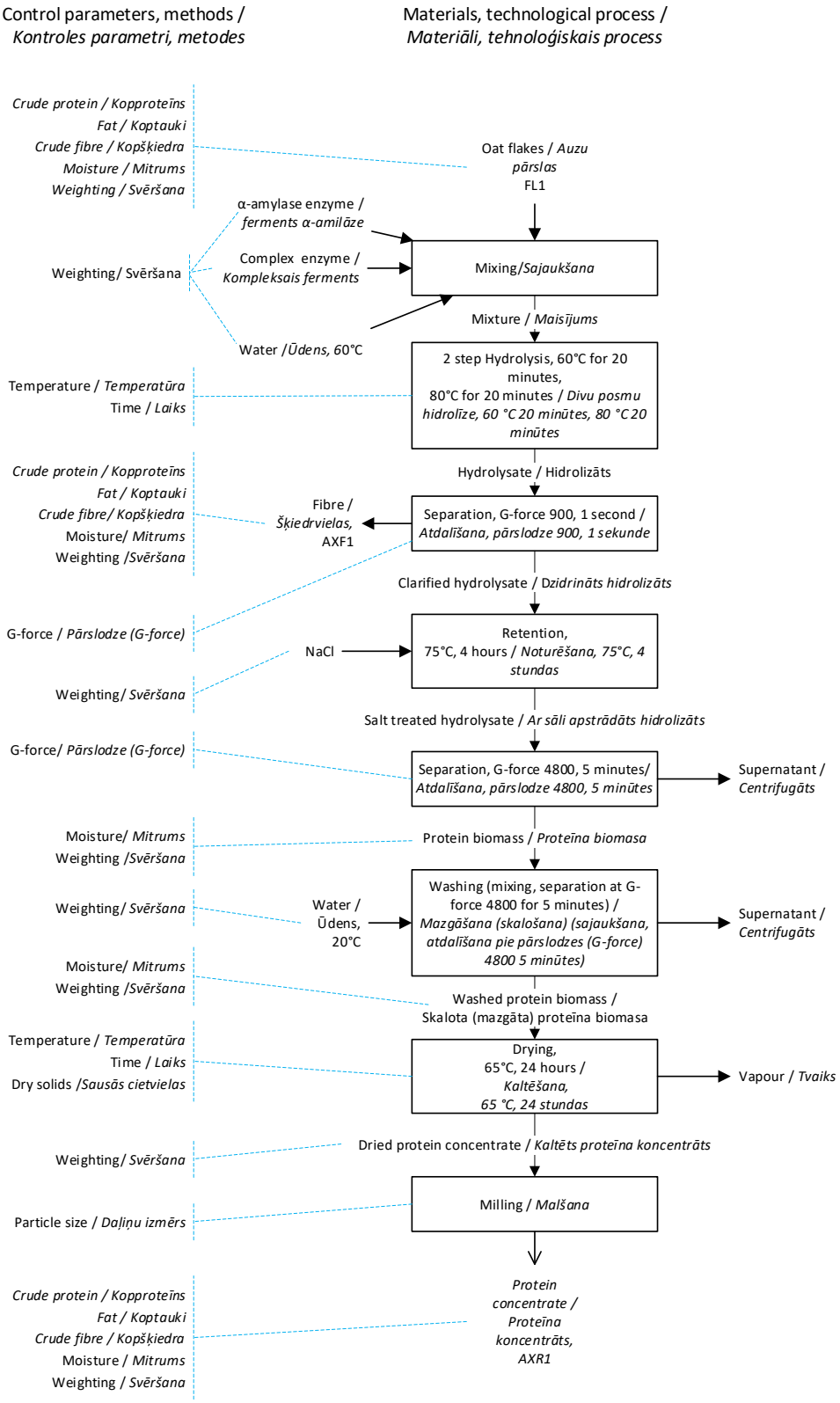


Scheme of oat protein extraction by α-amylase in 0.1 *M* NaCl solution

Control parameters and methods describe measurements and applied analysis used for the particular process

Auzu proteīna ekstrakcijas shēma ar a-amilāzi 0,1M NaCl šķīdumā

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

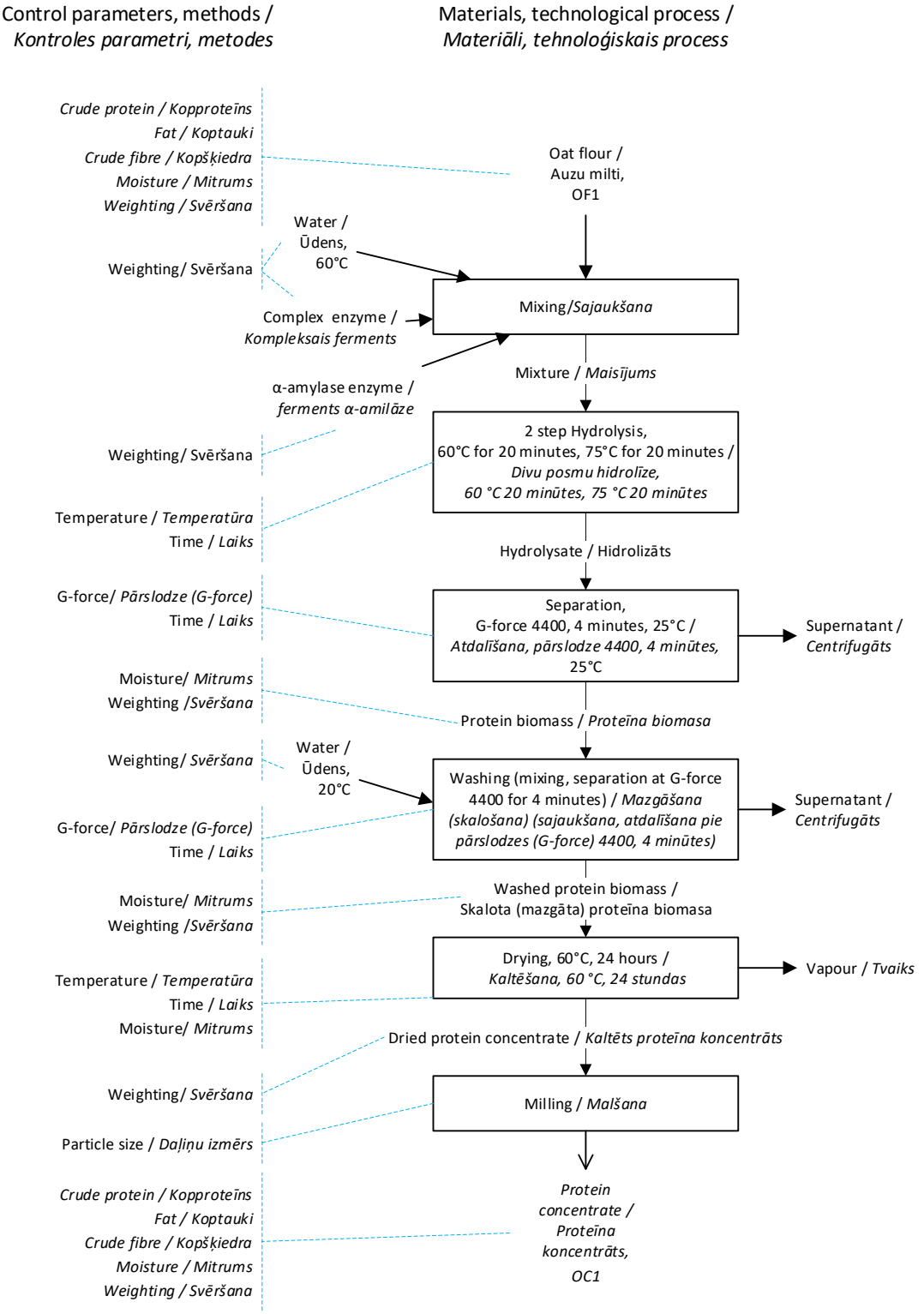


Scheme of oat protein extraction by α-amylase and complex enzymes for non-starch polysaccharides in 0.1 *M* NaCl solution

Control parameters and methods describe measurements and applied analysis used for the particular process.

Auzu proteīna ekstrakcijas shēma ar a-amilāzi un kompleksajiem fermentiem cieti nesaturošiem polisaharīdiem 0,1M NaCl šķīdumā

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

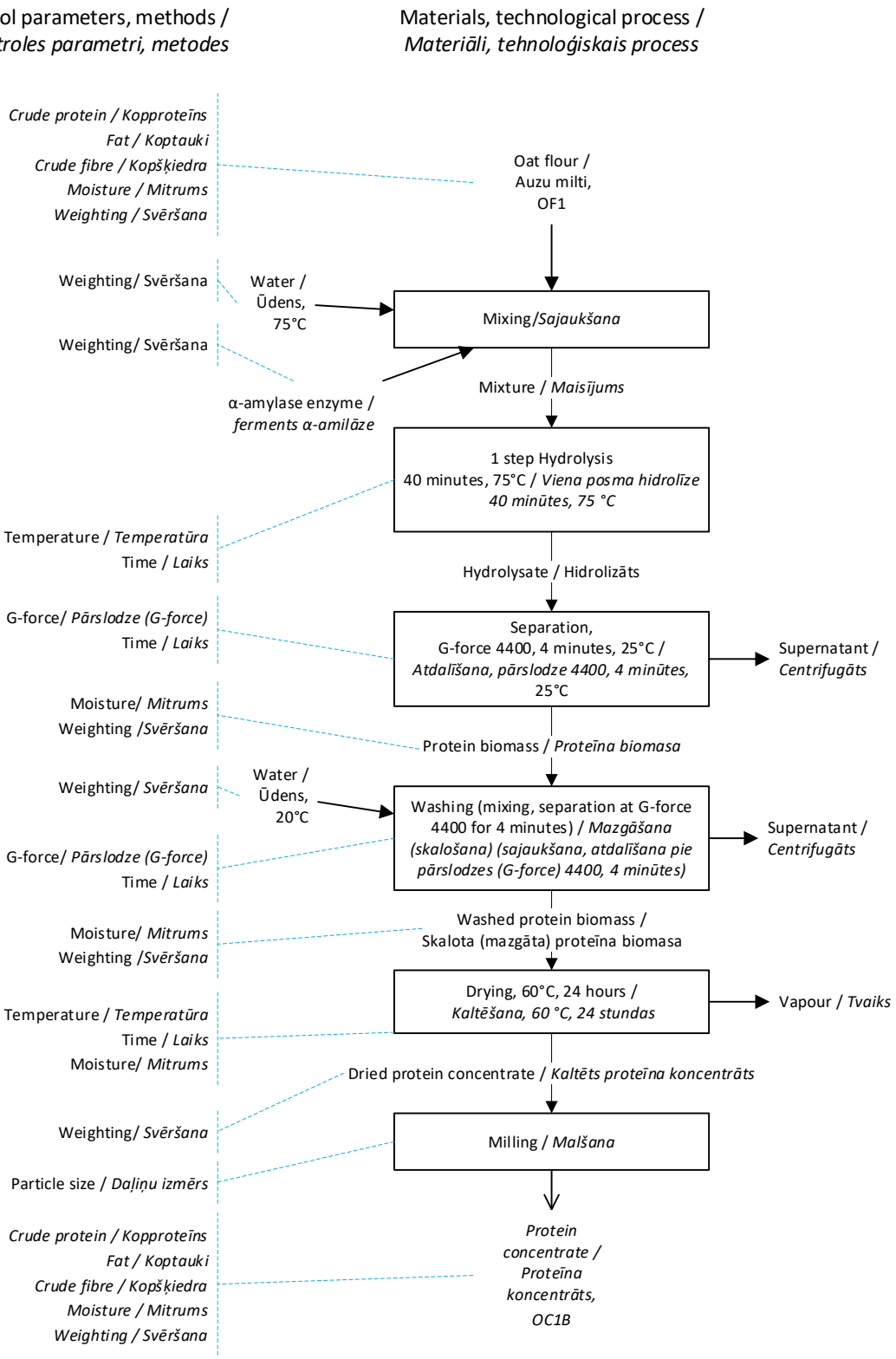


Scheme of obtaining oat protein concentrate in oat flour through enzymatic hydrolysis of starch and non-starch polysaccharides

Control parameters and methods describe measurements and applied analysis used for the particular process.

Auzu proteīna koncentrāta ieguves shēma auzu miltos, izmantojot cietes un necietes polisaharīdu fermentatīvo hidrolīzi

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

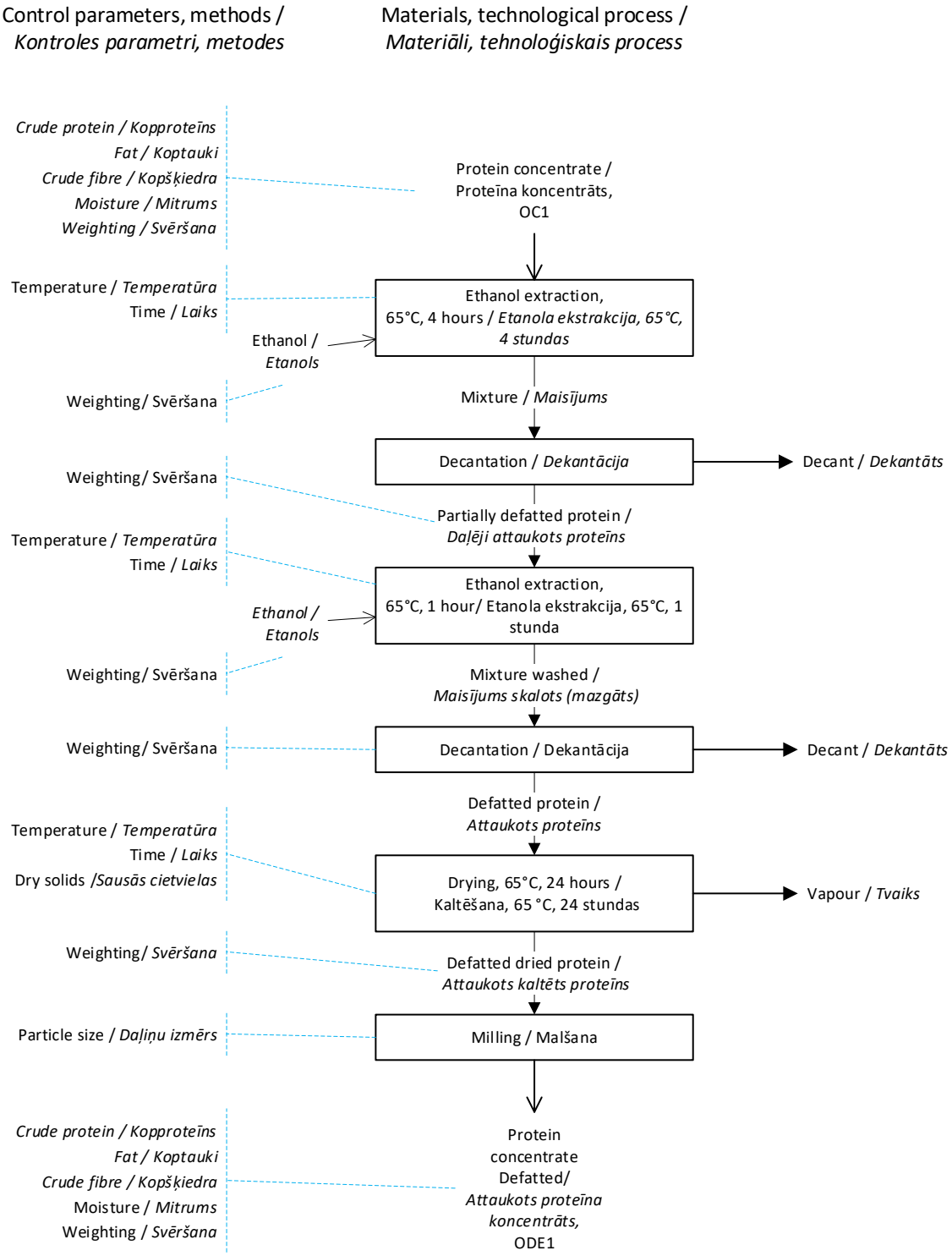


Scheme of obtaining oat protein concentrate by means of starch enzymatic hydrolysis in oat flour

Control parameters and methods describe measurements and applied analysis used for the particular process.

Auzu proteīna koncentrāta ieguves shēma, izmantojot auzu miltu cietes fermentatīvo hidrolīzi

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi

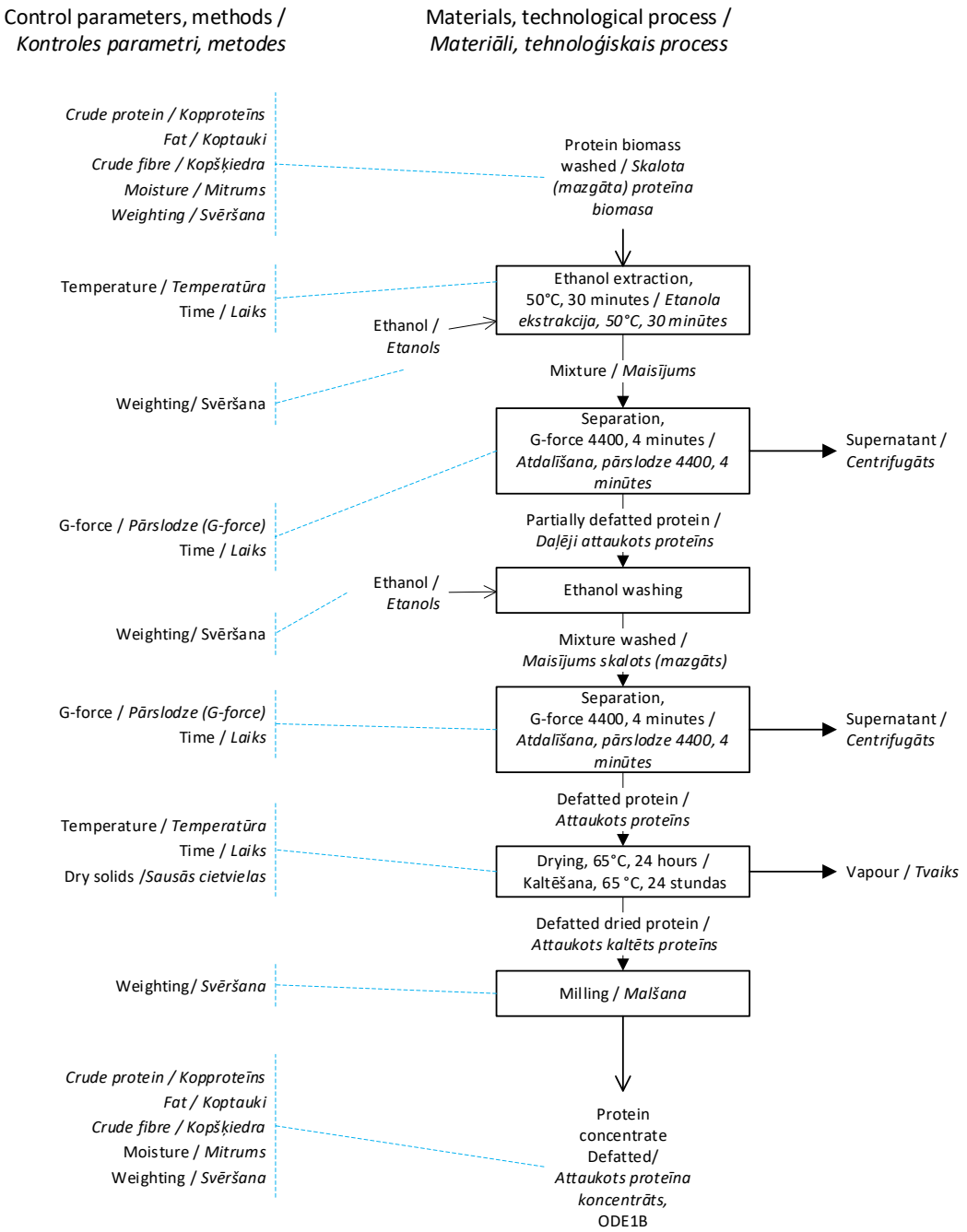


Scheme of oat protein concentrate defatting by ethanol

Control parameters and methods describe measurements and applied analysis used for the particular process

Auzu proteīna koncentrāta attaukošanas shēma ar etanolu

Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un piemēroto analīzi



Scheme of protein defatting in wet protein sample

*Control parameters and methods describe measurements and applied analysis used for the particular process. *Protein biomass washed is referred to in* ***the scheme illustrated in Appendix 6***

Proteīna attaukošanas shēma mitrā proteīna paraugā

*Kontroles parametri un metodes apraksta konkrētajā procesā izmantotos mērījumus un pielietotās analīzes. *Skalota (mazgāta) proteīna biomasa ir norādīta* ***6. pielikumā attēlotajā shēmā***