



This is a post-peer-review, pre-copyedit version of an article published in European Food Research and Technology. The final authenticated version is available online at: <https://doi.org/10.1007/s00217-020-03661-2>

Document downloaded from:



1 **Oat proteins as emerging ingredients for food formulation: where we stand?**

2 Fatma Boukid*

3 Institute of Agriculture and Food Research and Technology (IRTA), Food Safety Programme, Food
4 Industry Area, Finca Camps i Armet s/n, 17121 Monells, Catalonia, Spain

5 * Corresponding author: fatma.boukid@irta.cat

6

7

8 **Abstract:**

9 Over the last decades, interest in oats (*Avena sativa* L.) as healthy foods increased due to their multiple
10 functional and bioactive components such as dietary fibers, polyphenols, and proteins. Protein extracted
11 from oats received considerable attention being more abundant (12-20%) and having a distinct composition
12 compared to other cereal grains. Oat proteins also present pleasant sensorial attributes compared to proteins
13 deriving from legumes and oil seeds. Protein isolates or concentrates can be dry- or wet- extracted, and a
14 subsequent enzymatic hydrolysis can release peptides with bioactive properties. Several strategies have
15 been successfully applied to improve the techno-functionality of oat proteins. In term of food application,
16 there are few oat protein-based food products available in the market, which urge food developers to build
17 tailored strategies and food portfolios of these ingredients. This review will address oat proteins extraction
18 technologies and pre/post-treatment strategies, main characteristics, and applications. Future research is
19 still required to take advantage of breeding progress to select high grain-protein oat varieties and to boost
20 the incorporation of oat proteins in foods, while keeping in mind the cost and the environmental impact.

21

22 **Keywords:** oat protein, extraction, techno-functionality, health benefits, allergenicity

23

24

25

26 **1. Introduction**

27 Interests in protein-rich diet are rising favored by its health-promoting effects, with particular attention to
28 the source. Plant proteins are gaining interest as “better for you” and “better for the planet” alternatives to
29 animal proteins [1]. As a result, the market request to innovative non-animal plant sources keeps increasing,
30 where the main drivers are consciousness towards health, environment, and animal welfare. The urge to
31 search for alternative non-animal protein sources opened the way to the valorization of non-fully exploited
32 plants and industrial by-products. Oat (*Avena sativa* L.) is the sixth highest consumed type of cereal, with
33 a global annual production of 23 million tones, where Russia is the largest producer of this crop worldwide
34 [2].

35 Oats, wheat, barley and rye belong to the same *Poaceae* family, where oats are sub-classified into the
36 *Aveneae* tribe while the other cereals belong to the *Triticeae* tribe [3]. Table 1 illustrated oat grain
37 composition in comparison to other grains (cereals, legumes, and oil seeds) showing particularly high
38 amount of protein and lipids compared to rice and wheat. Beyond β -glucan production, oat has recently
39 attracted research and commercial attention as a superior cereal sources of low-cost dietary proteins (up to
40 20% protein) [4]. The distribution of proteins within oat grain is heterogeneous, following an increasing
41 gradient from the interior to the periphery, where proteins are located primarily in the germ and the bran
42 but less in the endosperm [5]. Like plant proteins, oat protein are industrially extracted using alkaline
43 extraction-isoelectric precipitation process [6, 7]. Dry fractionation was also suggested as a sustainable
44 process for plant protein production [8]. Moreover, oat protein concentrate can be recovered as a by-product
45 from β -glucan production thereby improving the sustainability of the process and contributing in circular
46 economy [9, 10]. Oat proteins are composed of globulins (70–80%) followed by albumins (1–12%),
47 prolamins (4–15%) and glutenins (<10%), unlike other cereals where prolamins are the major storage
48 proteins [11]. Compared to other cereals, oat proteins have higher content of essential amino acids such as
49 lysine [12].

50 **Table 1: Chemical composition of oat grains (g/ 100g on dry basis)**

	Oat	Rice	Wheat	Pea	Soy
Protein	12-20	7-10	11-15	23–31	36-40
Carbohydrates	69-76	73-80	55-69	28–65	20-30
Fiber	5-10	1-8	12-15	22-27	5-12
Lipid	5-18	1-2	1-2	1–2	18-20
Ash	1-2	1-2	1-2	2-4	5-6

Reference	[5, 13–16]	[5, 18]	[19, 20]	[19, 21, 22]	[23, 24]
	[17]				

51

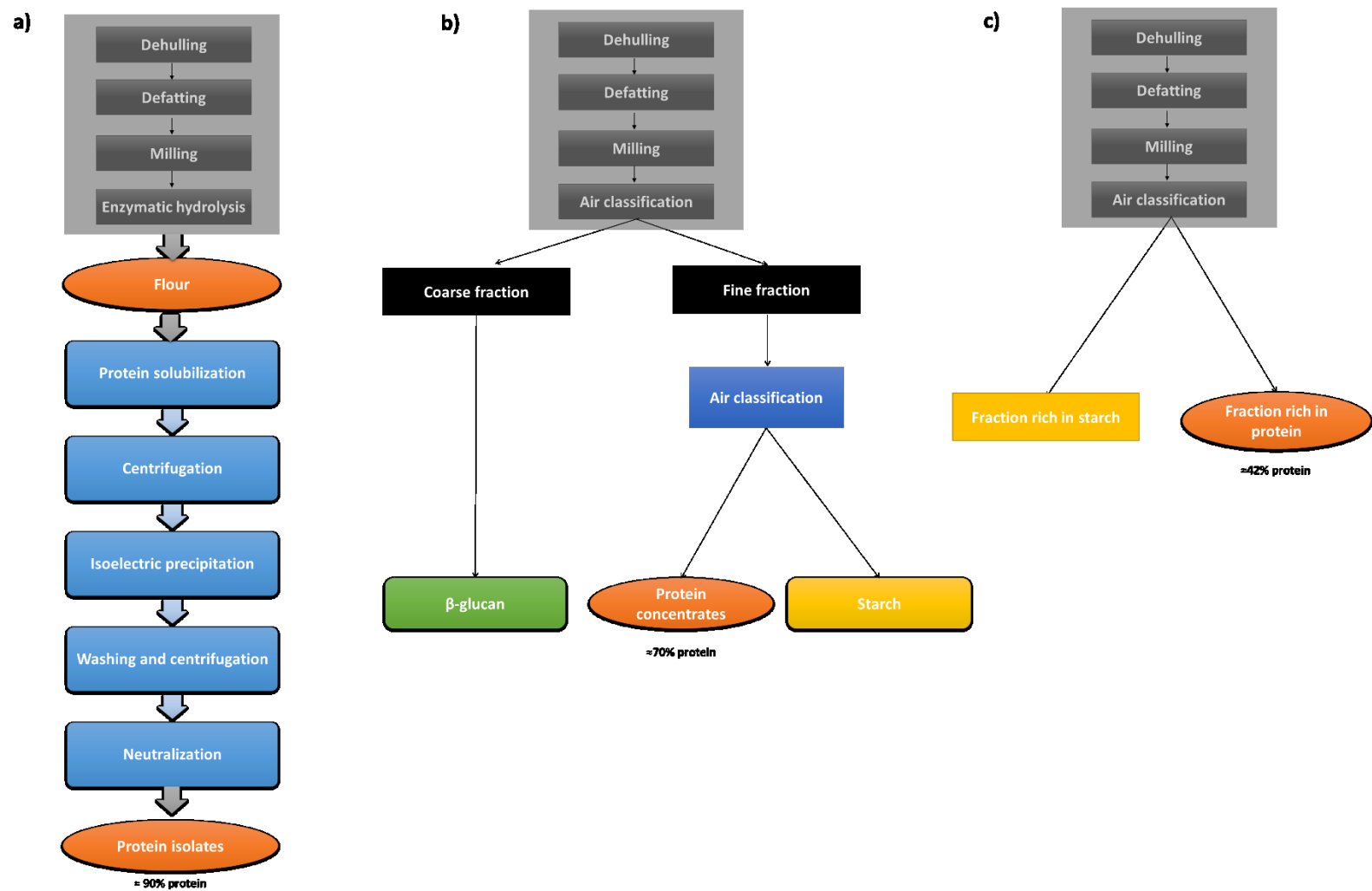
52 Plant proteins are gaining considerable interest as food ingredients with respect to the latest trends towards
53 a healthy lifestyle, flexitarianism, vegetarianism and veganism [9]. Global oat protein market is forecasted
54 to grow at a compound annual growth rate of 1.22% during the forecast period (2019-2024) [25]. These
55 proteins are highly accepted by consumers compared to other plant alternatives such as soy, pea and lupine
56 proteins since no off-flavors concerns are raised [10, 26]. From a sustainability standpoint, food products
57 containing oat proteins showed lower carbon footprint and land use than their counterparts made with
58 animal proteins [27]. Indeed, if 24% of animal-based food is replaced by oat protein concentrate-based
59 food, greenhouse gas emissions could be reduced by 8% and land use by 14% [28]. The techno-functionality
60 of oat proteins is still challenging, where low solubility, emulsifying capacity, foam ability and gel property
61 can limit their application in foods [29]. Therefore, appropriate chemical, physical and enzymatic methods
62 were developed to improve techno-functional and nutritional properties [30]. In the light of the
63 considerations, this review will give an updated compilation of oat proteins from basics to applications.

64 **2. Processing of oat proteins**

65 Oat grains or flours can go through pretreatments prior to extraction or fractionation to increase protein
66 yield. Heat treatment of oat grains ensured lipase inactivation (about 60%) thereby avoiding the
67 development of rancidity in oats during storage [31, 32]. This treatment also enhanced the extraction yield
68 [31, 32]. Dehulling (or pearling) is required for husked oat, where unpalatable hulls are removed by the
69 combined action of friction and abrasion using abrasive devices (Satake or tangential abrasive dehulling
70 device) [33]. A patented process for oat dehulling ensured the remove of 15% of grain weight with a prior
71 phased of grain steeping in an aqueous medium for up to 4 hours [34]. In the case of naked oats, varieties
72 with no hulls, hulls genes are removed allowing to reduce the production costs by avoiding the dehulling
73 step [35]. The dehulled or naked oat grains can be subjected to defatting with ethanol, hexane or
74 supercritical CO₂ extraction to remove lipids since oat has relatively high fat content (5 to 10%) [13–15].
75 Defatted oat grains can go through milling to produce oat flour. Prior to alkaline extraction, enzymatic
76 treatment with amyloglucosidase can increase the release of proteins during extraction thanks to the
77 breakdown of polysaccharides [36]. This enzymatic treatment can also enhance the techno-functionality of
78 proteins by increasing solubility, water retention and foaming capacity [4]. Sprouting process was also used
79 for boosting the bioactive and nutritional properties of oat-derived ingredients [37].

80 The alkaline method is the most commonly used method for extracting protein from oats [38]. The
81 extraction was performed at pH 9.2 to solubilize oat proteins, followed by isoelectric precipitation at pH 5,
82 washing with water, neutralization and subsequent drying to obtain oat protein isolates with up to 90%
83 protein (Fig. 1A) [4]. When the main purpose of extraction is β -glucan recovery (Fig. 1B), the denaturing
84 effect of high pH may be beneficial for protein solubilization contrary to the acidic extraction of oat β -
85 glucan [39]. Protein contents of oat protein concentrate can range from 69 to 90% [40]. Dry fractionation
86 (Fig. 1C) consists in milling oat grits or oat grains followed by air classification to obtain a fraction rich in
87 proteins containing 42% protein [41]. This mild method allows the retention of starch and lipid, which can
88 contribute in the physicochemical and tech-functional properties of oat protein concentrate [6]. In contrast
89 to dry methods, wet fractionation produces isolates with high protein purity and yield, yet it requires high
90 amounts of solvents and chemicals, which can potentially induce the loss of some health beneficial
91 components and changes in the structure of proteins[4, 42]. Moreover, this wet process requires high
92 water and energy consumption and generates high aqueous waste that can negatively impact the
93 environment [8].

94 Oat protein concentrate or isolates could be enzymatically hydrolyzed to produce bioactive peptides, which
95 can be further purified either by membrane or chromatographic methods to yield pure peptides [7]. Oat
96 proteins hydrolyzed with protease generate peptides with biological functions and improved functional
97 properties [43, 44], yet such a process can be time-consuming and expensive [45, 46]. Alternatively, partial
98 hydrolysis with alcalase can be used for protein hydrolysis, where it was reported to improve the functional
99 and antioxidant characteristics of oat protein isolate, particularly the hydrolysate with a moderate alcalase
100 hydrolysis (6%) [47]. Subsequent ultrasound treatment increased the efficiency of enzymatic hydrolysis by
101 increasing inhibitory activities of peptides against angiotensin converting enzyme [45].



102

103 **Figure 1:** Processing of oat proteins. a: Alkaline method [4]; b: Recovery of oat concentrate from β-glucan production chain [40]; c: Dry fractionation
 104 [6].

105 3. Structure and composition of oat proteins

106 Oat protein contains globulins (\approx 50–80%), albumins (\approx 1–12%), prolamins (\approx 4–15%) and glutelins (\approx 10%)
107 [48]. Globulins are salt-soluble proteins, and consist of three fractions including 12S globulin as major
108 fraction followed by 7S and 3S. Oat globulin (12S) is an oligomeric protein with six quaternary monomer
109 subunits (each 54–60 kDa), which resembles the structure of soy 11S globulin (glycinin) [49, 50]. The two
110 major subunits of 12S are A- and B-subunits, where A-subunit (32 kDa) is an acidic polypeptide and the
111 B-subunit (22 kDa) is a basic polypeptide [51]. The 7S globulins are polypeptides ranging from 55 kDa to
112 65 kDa. The 3S fraction is characterized by two polypeptides with molecular weights of about 15 and
113 21 kDa [46, 52]. Albumins (19 to 21 KDa) are mainly enzymes playing roles in the overall protein quality
114 and plant defense mechanism [53]. The alcohol-soluble prolamins fraction, avenins, consists of four
115 fractions α , β , γ , and ω -avenins [54]. Avenins have a low molecular weight (20–40 kDa) and have structural
116 homology to the sulphur-rich subgroup α -gliadins and γ -gliadins of wheat, the B-hordeins of barley, and
117 the γ -secalins of rye [55]. These proteins have a storage function similar to wheat gluten, but with different
118 amino acid composition (poor in proline and glutamine) [54]. Glutelins are polypeptides ranging from 10
119 to 90 kDa [53].

120 Amino acid composition of oat proteins was reported to vary significantly among the different protein
121 fractions obtained by Osborne method [53]. Globulins present the highest amounts of most essential amino
122 acids (*e.g.* phenylalanine, lysine, histidine and valine) and non-essential amino acids (*e.g.* glutamic acid,
123 arginine) compared to albumins, prolamins and glutelins [53]. Globulins essential amino acids composition
124 meets FAO-recommended values for adults for all amino acids except for methionine [56]. Based on the
125 ratio of essential amino acid content to the total amino acid content, both albumin (39%) and globulin (36%)
126 have values higher than 36%, and therefore, can be qualified as high-quality proteins unlike the prolamins
127 and glutelins fractions [56]. Beside the fractionation, the amino acid composition of protein fractions varied
128 importantly as function of used oat varieties according to variety (*i.e.* genetic) and also environmental
129 factors. The extraction method (temperature, pH and solvents) was also noted to impact the outcome [57].

130 To evaluate the quality of oat proteins in terms of the amino acid composition and quantity, Table 2 included
131 different types of proteins deriving from cereals (gluten-free and gluten containing), oil seeds and pulses.
132 Commercial oat proteins have comparable composition to that of rice and better than wheat, while they
133 showed low essential amino acid contents compared to soy and pea proteins [9, 58]. Oat is more abundant
134 in the sulphur-containing amino acids cysteine and methionine, compared to pulses [59]. Therefore, oat and
135 pulses proteins can be blended to have higher protein quality and specifically with greater lysine
136 concentrations to satisfy the daily human amino acids requirement [60]. Based on the ratio of essential

137 amino acid content to the total amino acid content, oat proteins can be categorized as high-quality proteins
 138 since the essential amino acid content to the total amino acid content ratio reached 36% as recommended
 139 by FAO [56]. Noteworthy, depending on the variety and the purity, oat proteins can show variable protein
 140 scores [60].

141 **Table 2:** Amino acid content of oat proteins (values are presented in g per 100 g of commercially available
 142 isolated protein powder) [58]

	Oat	Rice	Wheat	Pea	Soy	FAO standard (adult)
Protein content¹	64	79	81	80	91	
Essential amino acids ²						
Isoleucine	1.3	2	2	2.3	1.9	1.3
Leucine	3.8	5.8	5	5.7	5	1.9
Threonine	1.5	2	1.8	2.5	2.3	0.9
Phenylalanine	2.7	3.7	3.7	3.7	3.2	1.9
Lysine	1.3	1.9	1.1	4.7	3.4	1.6
Histidine	0.9	1.5	1.4	1.6	1.5	1.6
Valine	2	2.8	2.3	2.7	2.2	1.3
Methionine	0.1	2	0.7	0.3	0.3	1.7
ΣEAA	13.7	22.1	18	23.6	19.9	-
Non-essential amino acids						
Serine	2.2	3.4	3.5	3.6	3.4	-
Glutamic acid	11	12.7	26.9	12.9	12.4	-
Glycine	1.7	3.4	2.4	2.8	2.7	-
Alanine	2.2	4.3	1.8	3.2	2.8	-
Cysteine	0.4	0.6	0.7	0.2	0.2	-
Arginine	3.1	5.4	2.4	5.9	4.8	-
Proline	2.5	3.4	8.8	3.1	3.3	-
Tyrosine	1.5	3.5	2.4	2.6	2.2	-
ΣNEAA	24.7	36.8	48.9	34.4	31.9	-
ΣAA	38.4	58.9	66.9	58	51.8	-
ΣEAA/ ΣAA	36	37	27	41	38	36

143 ΣEAA sum of all essential amino acids, ΣNEAA sum of all non-essential amino acids, ΣAA sum of all amino acids,

144 ΣEAA/ ΣAA: the essential amino acid content to the total amino acid content ratio

145 ¹: determined using the Dumas combustion method.

146 ²: determined using ultra-performance liquid chromatography (UPLC) tandem mass spectrometry

147

148 4. Health benefits and concerns of oat proteins

149 In term of health benefits, several studies were conducted to determine the impact of oat protein and
 150 hydrolyzed oat proteins on human health (Table 3). The intake of oat protein increased swimming

151 endurance and the levels of liver glycogen, enhanced the activities of lactic dehydrogenase and superoxide
 152 dismutase and decreased the levels of blood urea nitrogen and malondialdehyde in serum [61]; This
 153 demonstrated the anti-fatigue function of oat protein but no clear indication was provided about the
 154 mechanism of action. Moreover, the daily consumption of oat protein (25 g) facilitated the recovery from
 155 exhaustive downhill running since it significantly inhibited limb edema following damaging exercise, and
 156 reduced the adverse effects on muscle strength, knee-joint range of motion, and vertical jump performance
 157 [62].

158 Oligopeptides deriving from hydrolyzed oat proteins were reported to have biological activities including
 159 antioxidant, anti-inflammatory and antihypertensive properties [7, 63, 64]. Oat proteins were found efficient
 160 in inhibiting renin (around 40.5%-70.9%) and enzyme angiotensin-I-converting enzyme (around 86.6%-
 161 96.5%) but poor relatively poor dipeptidyl peptidase-IV inhibition (around 3.7–46.3%) [63]. Based on *in*
 162 *silico* digestion, several peptide sequences (FFG, IFFFL, PFL, WWK, WCY, FPIL, CPA, FLLA, and
 163 FEPL) were identified as responsible of these inhibitory activities suggesting their potential use for treating
 164 hypertension and diabetes [63]. The antioxidant activity investigated using a cellular model was attributed
 165 to several factors including reduced production of intracellular reactive oxygen species, increased cellular
 166 glutathione, and increased activities of three main endogenous antioxidant enzymes [64]. Particularly, the
 167 peptides, LVYIL and YHNAPGLVYIL, were reported to be associated with increased activities of
 168 antioxidant enzymes with an increase of 29% in cell viability [64]. Antioxidant peptides (IRIPIL, FLKPMT,
 169 NSKNFPTL, LIGRPIIY, and FNDILRRGQLL) isolated from oat globulin hydrolyzed alcalase through
 170 ultra-filtration and ion-exchange chromatography showed bioactive effects [65]. These *in vitro* studies are
 171 promising but *in vivo* tests are required to identify antioxidant mechanisms of individual's peptides. The
 172 available *in vivo* studies focused on oat proteins or hydrolyzed and not on identifying specific high value
 173 peptides.

174 **Table 3: Health benefits of oat proteins**

Approach	Method	Impact	References
Animal study	male Kun-ming mice (n=30) were fed oat proteins (5.44 mg/g body weigh) dissolved in 2 mL distilled water daily for 30 days	increase swimming endurance and the levels of liver glycogen; enhance the activities of lactic dehydrogenase and superoxide dismutase; decrease the levels of blood urea nitrogen and malondialdehyde in serum	[61]

Human study	Healthy, untrained collegiate men (n=16) consumed oat protein (25 g protein) for 19 days	facilitate the recovery from exhaustive downhill running; ameliorate exercise-induced fatigue in mice; alleviate eccentric exercise induced skeletal muscle soreness; reduce the elevation of plasma IL-6 concentrations and serum creatine kinase, myoglobin and C reactive protein contents; inhibit limb edema following damaging exercise	[62].
In vitro study	Peptidomic approach of hydrolyzed oat proteins	antioxidant, anti-inflammatory and antihypertensive properties, and metal chelating properties	[7].
In silico digestion	The prediction of inhibitory peptides sequences from oat protein hydrolysates following in silico hydrolysis with the proteases, papain and ficin.	Inhibit the activity of renin, angiotensin-I-converting enzyme and dipeptidyl peptidase-IV	[63]
Cellular model	Human hepatocellular carcinoma (HepG2) cells were treated by isolated oat peptides	cytoprotection of cells; increase in cell viability	[64]
In vitro study	Determination of hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity	Identification of antioxidant peptides sequences: IRIPIL, FLKPMT, NSKNFPTL, LIGRPIIY, and FNDILRRGQLL	[65]

175

176 In the face of these health benefits, the safety of oat proteins for celiac patients remain controversial [3, 66–
177 68]. Several studies supported the safety of oat proteins for celiac patients [69, 70], while others reported
178 oat avenins as triggers to immune reaction in some cases of celiac patients [66, 71, 72]. This contradiction
179 might be explained by the high diversity in oat varieties (differing in prolamin genes) with different
180 immune-reactivity potentials [66, 67, 71]. According to the Codex Standard, CODEX STAN118-1979, oats
181 can be tolerated by most but not all people who are intolerant to gluten [73]. The contamination of oat with
182 gluten-containing grains is currently the main problem faced by people with celiac disease, where several
183 advanced analytical methods are developed to detect the presence of epitopes related to celiac disease in
184 blends of oat contaminated with others grains [69, 71, 74]. Based on EU regulation, oats and oat products
185 can be considered gluten-free if the maximum gluten contamination level do not exceed 20 ppm [75].

186 Therefore, establishing a separate gluten-free oat production chain requires controlling all steps in the chain
187 as well as labelling these products must contain indication on any potential contamination.

188 **5. Techno-functionality of oat proteins**

189 Oat protein have a poor solubility and poor emulsification properties due to the unfolding of oat globulins,
190 resulting in a transition from β -sheet to a random coil conformation and formation of insoluble aggregates
191 at pH between 3.0 and 7.0 [9]. This low solubility in acidic conditions limits the use of oat proteins in
192 dispersed food systems such as foams and emulsions [76]. Several strategies were applied to enhance the
193 techno-functionality of oat proteins as summarized in Table 4. Enzymatic treatment by trypsin, alcalase,
194 transglutaminase or glutaminase increased protein solubility, foaming properties and emulsification due to
195 the reduction of tertiary structure and molecular weight and the increase in the flexibility of protein
196 secondary structure and exposed hydrophobic side chains [29, 50, 76]. Chemical modification by acylation
197 was reported to decrease water holding capacity and increase emulsion activity index, compared with those
198 of native proteins [77]. Maillard reaction under controlled dry-heating conditions was reported to enable
199 the conjugation of oat protein with polysaccharides (*e.g. Pleurotus ostreatus* β -glucan and dextran), where
200 the resulting conjugates have better solubility, emulsifying properties and thermal stability compared to oat
201 protein isolate [12, 49].

202 Native oat proteins can form strong gels only at alkali pH with a heating phase (110–120 °C), while under
203 acidic and neutral pH, the formed gels are found weak with poor water holding capacity [46]. Partial
204 hydrolysis by flavourzyme and trypsin improved oat protein gel properties resulting in gel with comparable
205 mechanical strength to egg white protein at pH 9 [11, 46]. Both oat protein and its hydrolysate-based gels
206 exhibited excellent water-holding capacity at neutral or mildly alkaline conditions suggesting it potential
207 uses as a new and cost-effective gelling ingredient of plant origin to provide texture and structure in food
208 products [46]. Inulin addition at low concentrations (0.1–0.5%) resulted in strong oat protein gels at neutral
209 pH [11]. Cold-set gelation of oat protein consists in a heating step to enable proteins denaturation and then
210 polymerization followed by a cooling and the addition of Ca^{2+} or glucono- δ -lactone (GDL), resulting in the
211 formation of a soluble protein aggregates at ambient temperature [51, 78]. These cold-set oat protein isolate
212 gels could resist acidic juice and pepsin digestion thereby protecting both α -amylase enzyme activity and
213 the viability of probiotics in harsh gastric conditions [78]. Thus, these gels might be used as delivery
214 vehicles for sensitive compounds in food and non-food applications [78].

215 **Table 4: Post treatment for a better techno-functionality of oat proteins**

Treatment	Impact on structure	Impact on techno-functionality	Reference
Enzymatic treatment by trypsin, alcalase, transglutaminase or glutaminase.	-reduce tertiary structure and molecular weight -increase in the flexibility of protein secondary structure and exposed hydrophobic side chains	increase protein solubility, foaming emulsification, and gelling	[29, 50, 76] [11, 46]
Acylation	Formation of acyl linkage	decrease water holding capacity and increase emulsion activity index	[77]
Maillard reaction under controlled dry-heating conditions	to enable the conjugation of protein with polysaccharides (<i>e.g.</i> <i>Pleurotus ostreatus</i> β -glucan and dextran),	the resulting conjugates have better solubility, emulsifying properties and thermal stability compared to oat protein isolate	[12, 49]
Inulin addition	Formation of soluble aggregates	Enhance gelling	[11]
Addition of Ca²⁺ or glucono-δ-lactone	proteins denaturation resulting in the formation of a soluble protein aggregates	Enhance gelling	[51, 78]

216

217 6. Applications of oat proteins

218 The use of oat proteins is still limited in foods and beverages, yet the focus on improving the techno-
219 functionality might boost industrial incorporation oat proteins in foods. A recent study [79] investigated the
220 pretreatment of pea-oat protein blend by phytase and fermentation and subsequent extrusion cooking to
221 produce meat analogue. After texturization and cooking, the resulting meat analogue had improved
222 nutritional (reduction of antinutrients and increase in protein content and essential amino acids),

223 physicochemical (color and water/oil holding capacity) and textural properties (chewiness and resilience)
224 as well as flavor [79]. Concentrate of oat proteins were incorporated at three levels (1.1, 1.7 and 2.5%) in
225 fermented (*Lactobacillus delbrueckii* subsp. *bulgaricus* und *Streptococcus thermophilus*) yoghurt-product
226 [9]. The proteolytic enzymes present in the yoghurt culture cleaved oat proteins and released bioactive
227 peptides thereby enhancing the nutritional value of the product [9]. Furthermore, oat protein concentrates
228 (43% protein, 33% starch and 3.4% ash) enabled the increase of viscosity thanks to starch gelatinization
229 and resulted in a yoghurt combining nutritional benefits, sustainability and improved sensorial quality [10].
230 When oat protein isolates (90% oat protein and less than 1% starch) were used to make yoghurt, strong
231 sedimentation and high syneresis were induced due to the low oat protein functionality under acidic
232 conditions [10]. More work is required to provide a deeper knowledge into the role of starch and protein in
233 fermented oat protein concentrate based-yoghurts [9].

234 The use of oat protein for encapsulation may represent great opportunities for the consolidation of its
235 position in the market of food and nutraceutical products. Glycosylated oat protein (conjugated with β -
236 glucan) showed improved solubility, emulsifying capacity and thermo-stability compared to untreated oat
237 protein [12]. Given the poor bioavailability of β -carotene limits its utilization, β -carotene was encapsulated
238 by a conjugate formed by oat protein isolate and *Pleurotus ostreatus* β -glucan. Such conjugate protected
239 and stabilized β -carotene and increased its bioavailability and antioxidant activity [80]. Oat protein-shellac
240 combination gels also improved the bioavailability of resveratrol by protecting resveratrol along the
241 gastrointestinal tract of rats and improving its release in the intestinal environment, compared to free
242 resveratrol [81]. These studies suggest the potential use of oat proteins as a novel natural biopolymer
243 delivery system for bioactive compounds for food and biomedical applications [80, 81]. Future experiments
244 can focus on developing stable systems based on oat proteins at neutral pH [78].

245 7. Conclusion

246 In the frame of mapping new sources of alternative proteins, oat rises owing to the interesting properties of
247 its proteins compared to other cereal grains. Oat proteins have been launched in the market with a wide
248 spectrum of food and nutraceutical applications. As a functional food ingredient, the application of oat is
249 still in its early stages, where food companies are investing for producing high-quality oat proteins.
250 Noteworthy, native oat proteins present poor functional properties and consequently, attempts have been
251 made to improve their techno-functionality by chemical, physical and biological treatments. More
252 investigations are required to optimize and validate these processing steps to improve protein quality to
253 meet the food industry requirements. Safety, cost-efficiency, sustainability, bioactivity, and techno-
254 functionality are crucial features for the selection of processing and varieties to boost oat protein

255 applications. The contribution of breeding programs is of high relevance in positioning oat in the plant
256 protein market through the selection of high-quality protein varieties (up to 40% protein) [82].

257

258 **Acknowledgments**

259 This work was supported by CERCA Programme (Generalitat de Catalunya).

260

261 **References**

- 262 1. Boukid F (2020) Plant-based meat analogues: from niche to mainstream. *Eur Food Res Technol*
263 1:3. <https://doi.org/10.1007/s00217-020-03630-9>
- 264 2. FAO (2019) Oat production. <http://www.fao.org/home/en/>. Accessed 2 Sep 2020
- 265 3. Tanner G, Juhász A, Florides CG, et al (2019) Preparation and Characterization of Avenin-
266 Enriched Oat Protein by Chill Precipitation for Feeding Trials in Celiac Disease. *Front Nutr* 6:162.
267 <https://doi.org/10.3389/fnut.2019.00162>
- 268 4. Prosekov A, Babich O, Kriger O, et al (2018) Functional properties of the enzyme-modified
269 protein from oat bran. *Food Biosci* 24:46–49. <https://doi.org/10.1016/j.fbio.2018.05.003>
- 270 5. Beloshapka A, Buff P, Fahey G, Swanson K (2016) Compositional Analysis of Whole Grains,
271 Processed Grains, Grain Co-Products, and Other Carbohydrate Sources with Applicability to Pet
272 Animal Nutrition. *Foods* 5:23. <https://doi.org/10.3390/foods5020023>
- 273 6. Ramadhan K, Foster TJ (2018) Effects of ball milling on the structural, thermal, and rheological
274 properties of oat bran protein flour. *J Food Eng* 229:50–56.
275 <https://doi.org/10.1016/j.jfoodeng.2017.10.024>
- 276 7. Esfandi R, Willmore WG, Tsopmo A (2019) Peptidomic analysis of hydrolyzed oat bran proteins,
277 and their in vitro antioxidant and metal chelating properties. *Food Chem* 279:49–57.
278 <https://doi.org/10.1016/j.foodchem.2018.11.110>
- 279 8. Schutyser MAI, van der Goot AJ (2011) The potential of dry fractionation processes for
280 sustainable plant protein production. *Trends Food Sci. Technol.* 22:154–164
- 281 9. Brückner-Gühmann M, Vasil'eva E, Culetu A, et al (2019) Oat protein concentrate as alternative
282 ingredient for non-dairy yoghurt-type product. *J Sci Food Agric* 99:5852–5857.
283 <https://doi.org/10.1002/jsfa.9858>
- 284 10. Brückner-Gühmann M, Banovic M, Drusch S (2019) Towards an increased plant protein intake:
285 Rheological properties, sensory perception and consumer acceptability of lactic acid fermented,
286 oat-based gels. *Food Hydrocoll* 96:201–208. <https://doi.org/10.1016/j.foodhyd.2019.05.016>
- 287 11. Nieto-Nieto TV, Wang YX, Ozimek L, Chen L (2015) Inulin at low concentrations significantly
288 improves the gelling properties of oat protein - A molecular mechanism study. *Food Hydrocoll*
289 50:116–127. <https://doi.org/10.1016/j.foodhyd.2015.03.031>

- 290 12. Zhong L, Ma N, Wu Y, et al (2019) Characterization and functional evaluation of oat protein
 291 isolate-Pleurotus ostreatus β -glucan conjugates formed via Maillard reaction. *Food Hydrocoll*
 292 87:459–469. <https://doi.org/10.1016/j.foodhyd.2018.08.034>
- 293 13. Sterna V, Zute S, Brunava L (2016) Oat Grain Composition and its Nutrition Benefice. *Agric*
 294 *Agric Sci Procedia* 8:252–256. <https://doi.org/10.1016/j.aaspro.2016.02.100>
- 295 14. Stevenson DG, Inglett GE, Chen D, et al (2008) Phenolic content and antioxidant capacity of
 296 supercritical carbon dioxide-treated and air-classified oat bran concentrate microwave-irradiated in
 297 water or ethanol at varying temperatures. *Food Chem* 108:23–30.
 298 <https://doi.org/10.1016/j.foodchem.2007.08.060>
- 299 15. Walters M, Lima Ribeiro AP, Hosseinian F, Tsopmo A (2018) Phenolic acids, avenanthramides,
 300 and antioxidant activity of oats defatted with hexane or supercritical fluid. *J Cereal Sci* 79:21–26.
 301 <https://doi.org/10.1016/j.jcs.2017.09.010>
- 302 16. Hernot DC, Boileau TW, Bauer LL, et al (2008) In vitro digestion characteristics of unprocessed
 303 and processed whole grains and their components. *J Agric Food Chem* 56:10721–10726.
 304 <https://doi.org/10.1021/jf801944a>
- 305 17. Peterson DM, Wood DF (1997) Composition and structure of high-oil oat. *J Cereal Sci* 26:121–
 306 128. <https://doi.org/10.1006/jcrs.1996.0111>
- 307 18. Verma DK, Srivastav PP (2017) Proximate Composition, Mineral Content and Fatty Acids
 308 Analyses of Aromatic and Non-Aromatic Indian Rice. *Rice Sci* 24:21–31.
 309 <https://doi.org/10.1016/j.rsci.2016.05.005>
- 310 19. Boukid F, Zannini E, Carini E, Vittadini E (2019) Pulses for bread fortification: A necessity or a
 311 choice? *Trends Food Sci. Technol.* 88:416–428
- 312 20. Boukid F, Folloni S, Sforza S, et al (2018) Current Trends in Ancient Grains-Based Foodstuffs:
 313 Insights into Nutritional Aspects and Technological Applications. *Compr Rev Food Sci Food Saf*
 314 17:123–136. <https://doi.org/10.1111/1541-4337.12315>
- 315 21. Gularte MA, Gómez M, Rosell CM (2012) Impact of Legume Flours on Quality and In Vitro
 316 Digestibility of Starch and Protein from Gluten-Free Cakes. *Food Bioprocess Technol* 5:3142–
 317 3150. <https://doi.org/10.1007/s11947-011-0642-3>
- 318 22. Zare F, Champagne CP, Simpson BK, et al (2012) Effect of the addition of pulse ingredients to
 319 milk on acid production by probiotic and yoghurt starter cultures. *LWT - Food Sci Technol*
 320 45:155–160. <https://doi.org/10.1016/j.lwt.2011.08.012>
- 321 23. Wijewardana C, Reddy KR, Bellaloui N (2019) Soybean seed physiology, quality, and chemical
 322 composition under soil moisture stress. *Food Chem* 278:92–100.
 323 <https://doi.org/10.1016/j.foodchem.2018.11.035>
- 324 24. Silva F de O, Miranda TG, Justo T, et al (2018) Soybean meal and fermented soybean meal as
 325 functional ingredients for the production of low-carb, high-protein, high-fiber and high isoflavones
 326 biscuits. *LWT* 90:224–231. <https://doi.org/10.1016/j.lwt.2017.12.035>
- 327 25. Researchandmarkets Oat Protein Market - Growth, Trends and Forecasts (2019 - 2024). In: 2019.
 328 [https://www.researchandmarkets.com/reports/4622348/oat-protein-market-growth-trends-and-](https://www.researchandmarkets.com/reports/4622348/oat-protein-market-growth-trends-and-forecasts)
 329 forecasts. Accessed 26 Aug 2020
- 330 26. Brückner-Gühmann M, Benthin A, Drusch S (2019) Enrichment of yoghurt with oat protein
 331 fractions: Structure formation, textural properties and sensory evaluation. *Food Hydrocoll* 86:146–

- 332 153. <https://doi.org/10.1016/j.foodhyd.2018.03.019>
- 333 27. Heusala H, Sinkko T, Mogensen L, Knudsen MT (2020) Carbon footprint and land use of food
334 products containing oat protein concentrate. *J Clean Prod* 276:122938.
335 <https://doi.org/10.1016/j.jclepro.2020.122938>
- 336 28. Mogensen L, Heusale H, Sinkko T, et al (2020) Potential to reduce GHG emissions and land use
337 by substituting animal-based proteins by foods containing oat protein concentrate. *J Clean Prod*
338 274:122914. <https://doi.org/10.1016/j.jclepro.2020.122914>
- 339 29. Jiang Z qing, Sontag-Strohm T, Salovaara H, et al (2015) Oat protein solubility and emulsion
340 properties improved by enzymatic deamidation. *J Cereal Sci* 64:126–132.
341 <https://doi.org/10.1016/j.jcs.2015.04.010>
- 342 30. Zhao C Bin, Zhang H, Xu XY, et al (2017) Effect of acetylation and succinylation on
343 physicochemical properties and structural characteristics of oat protein isolate. *Process Biochem*
344 57:117–123. <https://doi.org/10.1016/j.procbio.2017.03.022>
- 345 31. Lehtinen P, Kiiliäinen K, Lehtomäki I, Laakso S (2003) Effect of heat treatment on lipid stability
346 in processed oats. *J Cereal Sci* 37:215–221. <https://doi.org/10.1006/jcrs.2002.0496>
- 347 32. Ziegler V, Ferreira CD, da Silva J, et al (2018) Heat-moisture treatment of oat grains and its
348 effects on lipase activity and starch properties. *Starch - Stärke* 70:1700010.
349 <https://doi.org/10.1002/star.201700010>
- 350 33. Wang R, Koutinas AA, Campbell GM (2007) Effect of pearling on dry processing of oats. *J Food*
351 *Eng* 82:369–376. <https://doi.org/10.1016/j.jfoodeng.2007.02.051>
- 352 34. Paton D, Reaney MJT, Tyler NJ (1999) US6113908A-Methods for processing oat groats and
353 products thereof
- 354 35. Antonini E, Lombardi F, Alfieri M, et al (2016) Nutritional characterization of naked and dehulled
355 oat cultivar samples at harvest and after storage. *J Cereal Sci* 72:46–53.
356 <https://doi.org/10.1016/j.jcs.2016.09.016>
- 357 36. Alrahmany R, Avis TJ, Tsopmo A (2013) Treatment of oat bran with carbohydrases increases
358 soluble phenolic acid content and influences antioxidant and antimicrobial activities. *Food Res Int*
359 52:568–574. <https://doi.org/10.1016/j.foodres.2013.03.037>
- 360 37. Aparicio-García N, Martínez-Villaluenga C, Frias J, Peñas E (2020) Changes in protein profile,
361 bioactive potential and enzymatic activities of gluten-free flours obtained from hulled and
362 dehulled oat varieties as affected by germination conditions. *LWT* 134:125914.
363 <https://doi.org/10.1016/j.lwt.2020.109955>
- 364 38. Guan X, Yao H (2008) Optimization of Viscozyme L-assisted extraction of oat bran protein using
365 response surface methodology. *Food Chem* 106:345–351.
366 <https://doi.org/10.1016/j.foodchem.2007.05.041>
- 367 39. Harasym J, Zyla E, Dziendzikowska K, Gromadzka-Ostrowska J (2019) Proteinaceous residue
368 removal from oat β -glucan extracts obtained by alkaline water extraction. *Molecules* 24:125914.
369 <https://doi.org/10.3390/molecules24091729>
- 370 40. Dawkins NL, Nnanna IA (1993) Oat Gum and β -Glucan Extraction from Oat Bran and Rolled
371 Oats: Temperature and pH Effects. *J Food Sci* 58:562–566. <https://doi.org/10.1111/j.1365-2621.1993.tb04324.x>

- 373 41. Sibakov J, Myllymäki O, Holopainen U, et al (2011) Lipid removal enhances separation of oat
374 grain cell wall material from starch and protein. *J Cereal Sci* 54:104–109.
375 <https://doi.org/10.1016/j.jcs.2011.04.003>
- 376 42. Liu K (2014) Fractionation of oats into products enriched with protein, beta-glucan, starch, or
377 other carbohydrates. *J Cereal Sci* 60:317–322. <https://doi.org/10.1016/j.jcs.2014.06.002>
- 378 43. Udenigwe CC, Gong M, Wu S (2013) In silico analysis of the large and small subunits of cereal
379 RuBisCO as precursors of cryptic bioactive peptides. *Process Biochem* 48:1794–1799.
380 <https://doi.org/10.1016/j.procbio.2013.08.013>
- 381 44. Cheung IWY, Nakayama S, Hsu MNK, et al (2009) Angiotensin-I converting enzyme inhibitory
382 activity of hydrolysates from oat (*Avena sativa*) proteins by in silico and in vitro analyses. *J Agric*
383 *Food Chem* 57:9234–9242. <https://doi.org/10.1021/jf9018245>
- 384 45. Wang B, Atungulu GG, Khir R, et al (2015) Ultrasonic Treatment Effect on Enzymolysis Kinetics
385 and Activities of ACE-Inhibitory Peptides from Oat-Isolated Protein. *Food Biophys* 10:244–252.
386 <https://doi.org/10.1007/s11483-014-9375-y>
- 387 46. Nieto-Nieto TV, Wang YX, Ozimek L, Chen L (2014) Effects of partial hydrolysis on structure
388 and gelling properties of oat globular proteins. *Food Res Int* 55:418–425.
389 <https://doi.org/10.1016/j.foodres.2013.11.038>
- 390 47. Zheng Z, Li J, Liu Y (2020) Effects of partial hydrolysis on the structural, functional and
391 antioxidant properties of oat protein isolate. *Food Funct* 11:3144–3155.
392 <https://doi.org/10.1039/c9fo01783f>
- 393 48. Boeck T, D'Amico S, Zechner E, et al (2018) Nutritional properties of various oat and naked oat
394 cultivars. *Bodenkultur* 69:215–226. <https://doi.org/10.2478/boku-2018-0018>
- 395 49. Zhang B, Guo X, Zhu K, et al (2015) Improvement of emulsifying properties of oat protein
396 isolate-dextran conjugates by glycation. *Carbohydr Polym* 127:168–175.
397 <https://doi.org/10.1016/j.carbpol.2015.03.072>
- 398 50. Nivala O, Mäkinen OE, Kruus K, et al (2017) Structuring colloidal oat and faba bean protein
399 particles via enzymatic modification. *Food Chem* 231:87–95.
400 <https://doi.org/10.1016/j.foodchem.2017.03.114>
- 401 51. Yang C, Wang Y, Chen L (2017) Fabrication, characterization and controlled release properties of
402 oat protein gels with percolating structure induced by cold gelation. *Food Hydrocoll* 62:21–34.
403 <https://doi.org/10.1016/j.foodhyd.2016.07.023>
- 404 52. Walters ME, Udenigwe CC, Tsopmo A (2018) Structural Characterization and Functional
405 Properties of Proteins from Oat Milling Fractions. *JAOCS, J Am Oil Chem Soc* 95:991–1000.
406 <https://doi.org/10.1002/aocs.12101>
- 407 53. Jing X, Yang C, Zhang L (2016) Characterization and Analysis of Protein Structures in Oat Bran.
408 *J Food Sci* 81:C2337–C2343. <https://doi.org/10.1111/1750-3841.13445>
- 409 54. Anderson OD (2014) The spectrum of major seed storage genes and proteins in oats (*Avena*
410 *sativa*). *PLoS One* 9:. <https://doi.org/10.1371/journal.pone.0083569>
- 411 55. Real A, Comino I, de Lorenzo L, et al (2012) Molecular and Immunological Characterization of
412 Gluten Proteins Isolated from Oat Cultivars That Differ in Toxicity for Celiac Disease. *PLoS One*
413 7:. <https://doi.org/10.1371/journal.pone.0048365>

- 414 56. WHO/FAO/UNU (2007) Protein and amino acid requirements in human nutrition - PubMed.
415 World Heal Organ Tech Rep Ser 935:1–265
- 416 57. Kriger O V., Kashirskikh E V., Babich OO, Noskova SY (2018) Oat protein concentrate
417 production. *Foods Raw Mater* 6:47–55. <https://doi.org/10.21603/2308-4057-2018-1-47-55>
- 418 58. Gorissen SHM, Crombag JJR, Senden JMG, et al (2018) Protein content and amino acid
419 composition of commercially available plant-based protein isolates. *Amino Acids* 50:1685–1695.
420 <https://doi.org/10.1007/s00726-018-2640-5>
- 421 59. Bonke A, Sieuwerts S, Petersen IL (2020) Amino Acid Composition of Novel Plant Drinks from
422 Oat, Lentil and Pea. *Foods* 9:429. <https://doi.org/10.3390/foods9040429>
- 423 60. Abelilla JJ, Liu Y, Stein HH (2018) Digestible indispensable amino acid score (DIAAS) and
424 protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in
425 20- to 30-kilogram pigs. *J Sci Food Agric* 98:410–414. <https://doi.org/10.1002/jsfa.8457>
- 426 61. Xu C, Lv J, You S, et al (2013) Supplementation with oat protein ameliorates exercise-induced
427 fatigue in mice. *Food Funct* 4:303–309. <https://doi.org/10.1039/c2fo30255a>
- 428 62. Xia Z, Cholewa JM, Dardevet D, et al (2018) Effects of oat protein supplementation on skeletal
429 muscle damage, inflammation and performance recovery following downhill running in untrained
430 collegiate men. *Food Funct* 9:4720–4729. <https://doi.org/10.1039/c8fo00786a>
- 431 63. Bleakley S, Hayes M, O’ Shea N, et al (2017) Predicted Release and Analysis of Novel ACE-I,
432 Renin, and DPP-IV Inhibitory Peptides from Common Oat (*Avena sativa*) Protein Hydrolysates
433 Using in Silico Analysis. *Foods* 6:108. <https://doi.org/10.3390/foods6120108>
- 434 64. Du Y, Esfandi R, Willmore W, Tsopmo A (2016) Antioxidant Activity of Oat Proteins Derived
435 Peptides in Stressed Hepatic HepG2 Cells. *Antioxidants* 5:39.
436 <https://doi.org/10.3390/antiox5040039>
- 437 65. Ma S, Zhang M, Bao X, Fu Y (2020) Preparation of antioxidant peptides from oat globulin. *CyTA*
438 - *J Food* 18:108–115. <https://doi.org/10.1080/19476337.2020.1716076>
- 439 66. Comino I, Bernardo D, Bancel E, et al (2016) Identification and molecular characterization of oat
440 peptides implicated on coeliac immune response. *Food Nutr Res* 60:.
441 <https://doi.org/10.3402/fnr.v60.30324>
- 442 67. Ahola HG, Sontag-Strohm TS, Schulman AH, et al (2020) Immunochemical analysis of oat
443 avenins in an oat cultivar and landrace collection. *J Cereal Sci* 95:103053.
444 <https://doi.org/10.1016/j.jcs.2020.103053>
- 445 68. Aaltonen K, Laurikka P, Huhtala H, et al (2017) The long-term consumption of oats in celiac
446 disease patients is safe: A large cross-sectional study. *Nutrients* 9:.
447 <https://doi.org/10.3390/nu9060611>
- 448 69. Fritz RD, Chen Y (2018) Oat safety for celiac disease patients: theoretical analysis correlates
449 adverse symptoms in clinical studies to contaminated study oats. *Nutr Res* 60:54–67.
450 <https://doi.org/10.1016/j.nutres.2018.09.003>
- 451 70. Pinto-Sánchez MI, Causada-Calo N, Bercik P, et al (2017) Safety of Adding Oats to a Gluten-Free
452 Diet for Patients With Celiac Disease: Systematic Review and Meta-analysis of Clinical and
453 Observational Studies. *Gastroenterology* 153:395-409.e3.
454 <https://doi.org/10.1053/j.gastro.2017.04.009>

- 455 71. Comino I, Real A, De Lorenzo L, et al (2011) Diversity in oat potential immunogenicity: Basis for
456 the selection of oat varieties with no toxicity in coeliac disease. *Gut* 60:915–922.
457 <https://doi.org/10.1136/gut.2010.225268>
- 458 72. Hardy MY, Tye-Din JA, Stewart JA, et al (2015) Ingestion of oats and barley in patients with
459 celiac disease mobilizes cross-reactive T cells activated by avenin peptides and immuno-dominant
460 hordein peptides. *J Autoimmun* 56:56–65. <https://doi.org/10.1016/j.jaut.2014.10.003>
- 461 73. Codex alimentarius commission (2015) Codex Stan 118-1979. Standard for Foods for Special
462 Dietary Use for Persons Intolerant to Gluten
- 463 74. Koerner TB, Cl  roux C, Poirier C, et al (2011) Gluten contamination in the Canadian commercial
464 oat supply. *Food Addit Contam - Part A Chem Anal Control Expo Risk Assess* 28:705–710.
465 <https://doi.org/10.1080/19440049.2011.579626>
- 466 75. European Commission (2014) Commission Implementing Regulation (EU) No 828/2014 on the
467 requirements for the provision of information to consumers on the absence or reduced presence of
468 gluten in food. *Off J, L228*, p 4
- 469 76. Br  ckner-G  hmann M, Heiden-Hecht T, S  zer N, Drusch S (2018) Foaming characteristics of oat
470 protein and modification by partial hydrolysis. *Eur Food Res Technol* 244:2095–2106.
471 <https://doi.org/10.1007/s00217-018-3118-0>
- 472 77. Mirmoghtadaie L, Kadivar M, Shahedi M (2009) Effects of succinylation and deamidation on
473 functional properties of oat protein isolate. *Food Chem* 114:127–131.
474 <https://doi.org/10.1016/j.foodchem.2008.09.025>
- 475 78. Yang C, Wang Y, Lu L, et al (2018) Oat protein-shellac beads: Superior protection and delivery
476 carriers for sensitive bioactive compounds. *Food Hydrocoll* 77:754–763.
477 <https://doi.org/10.1016/j.foodhyd.2017.11.017>
- 478 79. Kaleda A, Talvistu K, Tamm M, et al (2020) Impact of Fermentation and Phytase Treatment of
479 Pea-Oat Protein Blend on Physicochemical, Sensory, and Nutritional Properties of Extruded Meat
480 Analogs. *Foods* 9:1059. <https://doi.org/10.3390/foods9081059>
- 481 80. Zhong L, Ma N, Wu Y, et al (2019) Gastrointestinal fate and antioxidation of β -carotene emulsion
482 prepared by oat protein isolate-Pleurotus ostreatus β -glucan conjugate. *Carbohydr Polym* 221:10–
483 20. <https://doi.org/10.1016/j.carbpol.2019.05.085>
- 484 81. Yang C, Wang Y, Xie Y, et al (2019) Oat protein-shellac nanoparticles as a delivery vehicle for
485 resveratrol to improve bioavailability in vitro and in vivo. *Nanomedicine* 14:2853–2871.
486 <https://doi.org/10.2217/nmm-2019-0244>
- 487 82. Jackson EW (2017) US20170105379 High Protein Oat Species.
488 <https://www.freepatentsonline.com/20170105379.pdf>. Accessed 15 Nov 2020

489
490
491

492 **Figure caption:**

493 **Figure 1:** Processing of oat proteins. a: Alkaline method [4]; b: Recovery of oat concentrate from β -glucan
494 production chain [40]; c: Dry fractionation [6]. This figure illustrates the three potential processing enabling
495 the production of oat protein ingredients (isolates or concentrates)

496

497 **Table caption**

498 **Table 1:** Amino acid content of oat proteins (values are presented in g per 100 g oat proteins) [58]. This
499 table summarizes oat protein quantity and amino acid composition of oat proteins in comparison with
500 different protein grains.

501