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1 **Grain Proteins: challenges and solutions in developing consumer-relevant** 2 **foods**

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12 **Abstract:**

13 Grain proteins are gaining popularity as an animal-free alternative for food and beverages formulations.
14 Soy protein, gluten, zein and rice protein are the most used proteins deriving from cereals and pulses.
15 The shift from animal protein to plant proteins is driven by the increasing awareness about the health
16 benefits of these alternative proteins and concerns over animal welfare and sustainability. As a result,
17 grain proteins are trending upward as functional ingredients in food and beverage formulations. Some
18 challenges are mainly related to the beany flavor or lower functionality compared to animal proteins.
19 Several innovative strategies are developed to mitigate off-flavors and enhance functionality particularly
20 when grain proteins are used to substitute animal proteins in animal-free products: Food developers
21 modulate formulations and processing conditions to increase grain protein content to enhance nutritional
22 quality without hampering finished product quality More research is required to fully elucidate the
23 functionality of grain proteins and to select protein source and quantity for developing healthy and tasty
24 protein rich foods.

25

26 **Keywords:** grain proteins, extraction, functionality, food formulation, cereals, legumes

27 **1. Introduction**

28 Plant protein markets are witnessing a fast growth and are projected to keep growing at a compounded
29 annual growth rate of 8.1% from 2019 to 2025, with North America holding the largest share of the
30 market (Meticulous Research®, 2019). This expansion is driven by increased consumer awareness of
31 health- and wellness-consciousness, ethical concerns over animal welfare and sustainability, as well as
32 in response to growing populations (Akaichi, Glenk, and Revoredo-Giha 2019; van der Weele et al.
33 2019). Grain proteins hold the largest share of the plant protein market, particularly those derived from
34 cereals (wheat, rice and corn) and legumes (soy and pea) (Meticulous Research®, 2019a). These proteins
35 are gaining more popularity with emphasis on their compositional and functional properties for the
36 development of high-quality, consumer-relevant foods (Burger and Zhang 2019; Lammi, Zanoni, and
37 Arnoldi 2015; Hu et al. 2015). Nevertheless, the incorporation of grain protein is still challenging and
38 might induce changes to the quality of food products particularly when used as an alternative to animal
39 proteins or in animal-free products (Schreuders et al. 2019; Pietsch et al. 2019). In this light, this review
40 aims to define the characteristics of grain proteins and to address the challenges and solutions for
41 developing consumer-relevant foods.

42

43 **2. Extraction**

44 Wet extraction and dry fractionation are both employed for protein isolation. At industrial level, grain
45 protein isolates are traditionally extracted using wet processing. As illustrated in Figure 1, wet extraction
46 starts by subjecting finely milled (and defatted, dehulled or/ and debranned, depending on the grain type)
47 flour to alkaline or acidic conditions to solubilize proteins (Adenekan et al. 2018). After centrifugation,
48 for the removal of insoluble material (e.g., starch and fiber), the solubilized protein are/may be
49 concentrated by isoelectric precipitation, washed and centrifuged again to remove soluble material
50 (sugars, soluble fibers and fats). Proteins are then neutralized and dried to obtain proteins isolates with
51 high purity (90%) (Taherian et al. 2011; Papalamprou, Doxastakis, and Kiosseoglou 2010). However,
52 the use of chemical solvents and thermal treatments in this process may affect protein functionality
53 through hindering their structure (Jafari et al. 2016; Zhao et al. 2018). In addition, this process requires
54 high amounts of water and energy and generates high waste (products? biomass?), which could
55 negatively affect the environment (Ruiz et al. 2016; Chéreau et al. 2016). To overcome both functional
56 and environmental drawbacks, several innovative pre- and/or post-processing techniques have been
57 developed (Table 1).

58

59 **3. Characteristics of grain proteins**

60 Cereals are valuable sources of proteins in the human diet. Gluten, zein, and rice protein are the most
61 used proteins in food and beverage formulations owing to their techno-functional properties. Gluten has
62 a high concentration of sulfur-containing amino acids and plays an important role in water absorption
63 capacity, cohesiveness, viscosity, and elasticity of the dough (Ortolan et al. 2017). However, for those
64 consumers genetically predisposed, gluten is related to a wide spectrum of diseases such as celiac disease
65 and gluten sensitivity (Sapone et al. 2012). Rice proteins are an excellent source of essential amino
66 acids, whereas their native form have low solubility and emulsifying properties (Gomes and Kurozawa
67 2020). Rice proteins are hypoallergenic and rich in bioactive peptides (Amagliani et al. 2019). Zein is
68 the commercialized form of corn proteins, and it is rich in sulfur-containing amino acids, but lacks
69 tryptophan and lysine (Dhillon et al. 2016). Zein has a low solubility but high capacities in emulsion
70 and foam stability and film forming (Cao et al. 2020; Boostani et al. 2019; Teklehaimanot and
71 Emmambux 2019). As for legumes, soy protein is the most marketed protein and provides a relatively
72 well-balanced amino acid composition and bioactive peptides (Gorissen et al. 2018). Soy proteins
73 provide high gel formation, emulsifying capacity, solvent holding capacity and film forming capacities
74 (Li et al. 2019; Barac et al. 2015). Pea protein use is one the rise as a less allergenic alternative to soy
75 protein, offering good emulsification and foaming properties (Silva et al. 2019). More grain sources of
76 proteins are gaining traction including sweet lupin proteins and fava proteins.

77

78 **4. Enriching Foods with Grain Proteins: opportunities and challenges**

79 In meat analogues applications, soy and gluten play crucial roles in creating fibrous structure thanks to
80 their binding and film-forming capacities Pea proteins are increasingly used as a substitute for soy
81 protein, but this results in weaker structure thereby requiring the addition of other ingredients (*e.g.*
82 gluten) to reinforce and stabilize the fibrous structure (Schreuders et al. 2019).

83 Alternative dairy-free beverages enriched with soy and pea proteins are characterized by a distinct grassy
84 or beany flavor (Sethi, Tyagi, and Anurag 2016; Trikusuma, Paravisini, and Peterson 2020). The
85 addition of grain proteins can increase total protein and amino acid contents in beverages (Akin and
86 Ozcan 2017). More investigations are required to modulate grain proteins behavior as a function of
87 processing conditions to ensure quality stability during storage (Le Roux et al. 2020).

88 In bread, vital gluten is commonly added to weak wheat flour to improve the strength of the protein
89 network in the dough thereby enhancing the properties of the bread, including yield, volume, texture,
90 color, and sensory properties (Boukid et al. 2018). The incorporation of non-gluten proteins at up to

91 10% has been found to improve both the protein quantity and quality of bread, but over 15% weakened
92 the gluten-network of doughs and hindered bread quality (Hoehnel et al. 2019; Zhou, Liu, and Tang
93 2018). In gluten-free breads, the addition of protein at up to 2% enhanced dough rheological properties
94 and bread quality (*i.e.* specific volume, sensory quality, nutritional quality, and digestibility) (Masure
95 et al. 2019; Sahagún et al. 2020). However, protein additions at over 10% resulted in breads with darker
96 color, lower volume and higher hardness than control (Sahagún and Gómez 2018).

97 Gluten-free pasta enriched with proteins grains (up to 10%) had enhanced structure, texture, cooking
98 quality and sensory properties and reduced the digestibility of the final product (Linares-García et al.
99 2019; Detchewa et al. 2016; Rachman et al. 2019; Larrosa et al. 2016; Phongthai et al. 2017). However,
100 over 12% of zein resulted in the increase of water absorption and firmness (Jeong et al. 2017).

101 Baked goods made by substituting up to 30% of wheat flour by grain proteins resulted in the increase of
102 water absorption and expansion factor (Tang and Liu 2017), where up to 15% did not hinder hardness
103 and overall acceptability (Tang and Liu 2017). Likewise, gluten-free biscuits formulated by the
104 substitution of rice flour by soy protein or pea protein at up to 20% were well accepted by consumers,
105 but higher levels (over 20%) hampered the quality of biscuits (dark color and hard texture) thereby
106 lowering the overall acceptability of the enriched products (Adeyeye, Adebayo-Oyetero, and Omoniyi
107 2017; Mancebo, Rodriguez, and Gómez 2016).

108 To conclude, while plant proteins are becoming a desirable choice for consumers and food
109 manufacturers, there are still challenges that arise when using these ingredients. Breakthroughs in
110 understanding off-flavor mechanisms and new manufacturing techniques will breed the next generation
111 of ingredients that can give a higher quality compared to the analogs that are made with animal-based
112 or less amounts of protein. These improvements can lead the way in furthering consumer acceptance
113 leading to more demand for additional plant-based products.

114

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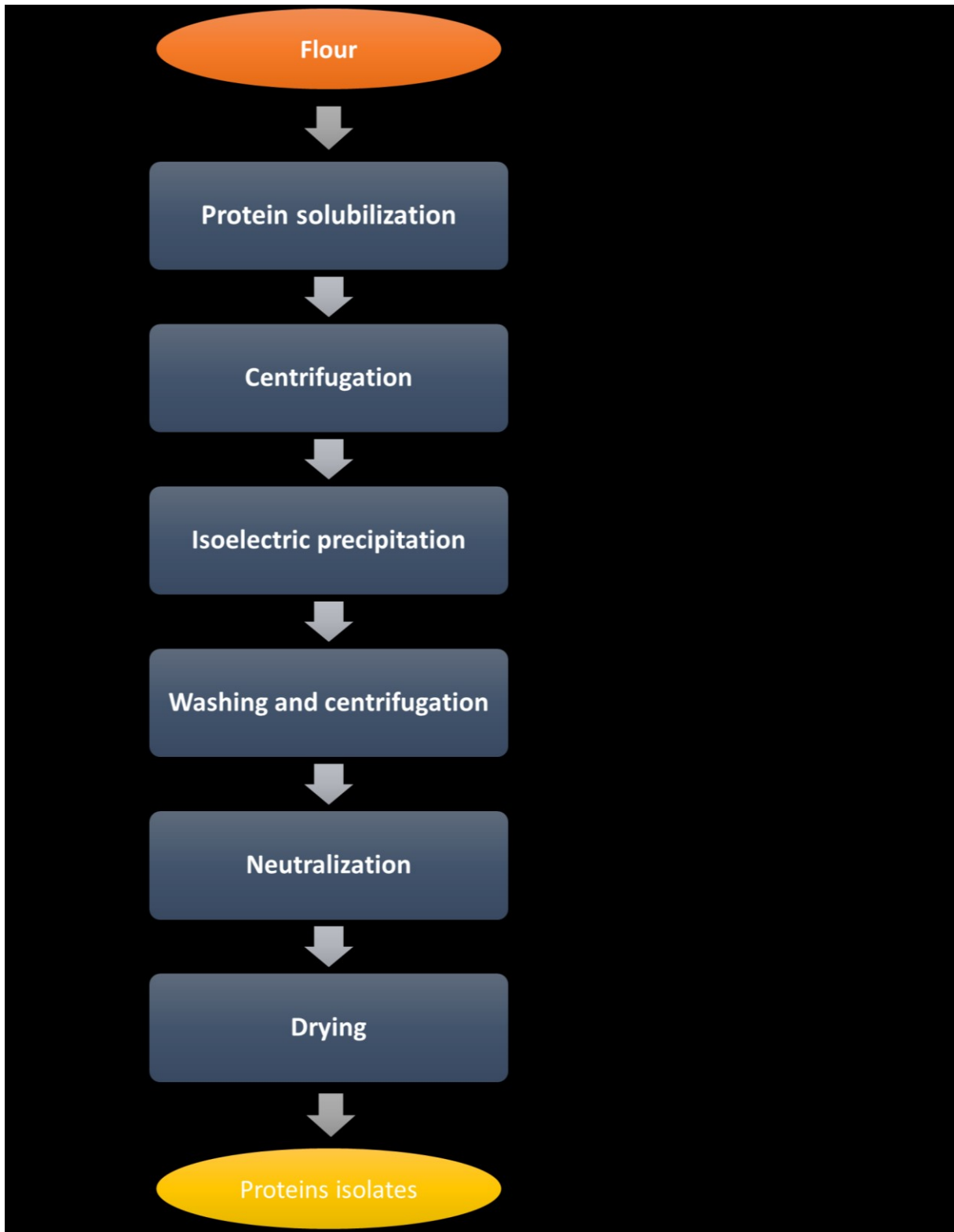
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331 **Figure 1:** Wet extraction of grain protein (Taherian et al. 2011; Papalamprou, Doxastakis, and
332 Kiosseoglou 2010).

333 **Table 1:** Innovative processing for grain proteins purification

Processing	Advantages	References
Electro-acidification and ultrafiltration	-improve protein purity -improve protein isolates solubility	(Mondor et al. 2004).
Single frequency ultrasound	improve protein isolates solubility and emulsifying capacity and stability	(Ma et al. 2020; Huang et al. 2020)
Multi-frequency ultrasonic	-improve protein structure and functionality -reduce extraction time	(Salimi Khorshidi et al. 2019; Yang et al. 2017; Golly et al. 2020)
Supercritical carbon dioxide	-green technology -remove bitterness and carotenoids compounds -lighten the color of protein	(Vatansever and Hall 2020)
Enzymatic extraction assisted with microwave or vacuum processing	-improve protein functionality -bioactivity of antioxidant compounds	(Görgüç, Özer, and Yılmaz 2020)
Lactic acid fermentation	-decrease flavor -reduce antinutrients -increase bioactive peptides	(Youssef et al. 2016; Çabuk et al. 2018)
Solvent treatment	-enhance the structure -decrease flavor retention	(Wang & Arntfield, 2015).
Acetylation and succinylation	- enhance the structure -decrease flavor retention	(Wang and Arntfield 2016)

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336 **Author biosketches**

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