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REVIEW

Replacement of animal proteins in food: How to take advantage of nutritional and gelling properties of alternative protein sources

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ABSTRACT

Given the growing world population, there is a need to balance animal and vegetable sources of dietary protein and to limit overall protein resources, and food formulation has to consider alternative protein sources as a way to meet human requirements. The protein concentration, essential amino acids (EAA) of all protein sources were analyzed with respect to human needs along with additional macronutrients of nutritional and energy interest (*i.e.* carbohydrates and lipids). New indexes are proposed to classify the alternative protein sources considering their EAA balance and how it may change during food processing. A global overview of all protein sources is provided including the quantity of food and associated caloric intakes required to fulfill our daily protein needs. As texture is a key parameter in food formulation, and is often influenced by protein gelation, we conducted an exhaustive review of the literature in a large scientific database on the ability of proteins from all sources to go through the sol-gel transition with the corresponding physical-chemical conditions. Traditional and innovative recipes are discussed and some improvement are proposed in terms of their ability to fulfill human needs for EAA and food and caloric intakes.

KEYWORDS

diet; essential amino acids; formulation; gelation; nutrition

Introduction

Proteins from animal sources are of great interest for their nutritional and functional properties. They are widely used by industry either to produce simple food products, such as steak or milk, with minimum modifications, or to produce formulated food products such as yoghurt, cheese, or surimi. From a nutritional point of view, animal proteins are appreciated for their quantity, high quality and digestibility, and their good balance of essential amino acids (EAA) necessary to synthetize body proteins (FAO (Food and Agriculture Organisation) 2009). From a functional point of view, animal proteins are known for their thickening, emulsifying and/or gelling properties, which make them useful to create textures of sensorial and practical interest (Strixner and Kulozik 2011).

However, livestock production has a considerable impact on the environment, contributing to soil erosion, water pollution, deforestation, desertification, greenhouse gas production and global warming (FAO (Food and Agriculture Organisation) 2006, 2009). The consumption of meat and processed meat is also a public health issue since it contributes to chronic diseases including cancers, diabetes and cardiovascular diseases (Walker et al. 2005). Other health issues concern the ingestion of pathogens responsible for chemical zoonosis (Pépin, Russo, and Pardon 1997) as well as chemicals such as antibiotics or hormones (Walker et al. 2005).

On the other hand, it is crucial to increase protein production to meet the growing demand due to global

population increase, and economic and nutritional transitions. The world population and caloric intake are expected to increase by, respectively, 6 billion and 500 kcal per day per capita between 2000 and 2100, accompanied by an increase in the proportion of animal products in the diet (FAO (Food and Agriculture Organisation) 2012b). As a result, by 2100, the global protein demand is expected to increase by 30% *i.e.* 10% per capita. In the absence of dietary changes, intense pressure will be exerted on environmental resources (Henchion et al. 2017; Tilman and Clark 2014). Thus to meet the increasing protein demand without harming the environment, it is crucial to study alternative sources of protein such as plants, insects, algae and mushrooms (Van Huis 2015). Creating novel foods using alternative protein sources or replacing proteins from animal sources in standard foods are options to be explored.

As the nutritional and functional properties of proteins vary, it can be challenging to obtain food products with targeted nutritional and sensory properties. Indeed, proteins are already known and exploited for their emulsifying, thickening or gelling properties, which are expressed in specific physical-chemical conditions. As gelation is often used to give texture to food products containing animal proteins, here we focus on this property. Essential amino acid (EAA) profiles and the digestibility of proteins from alternative sources are often described as less balanced and lower than those of animal proteins (Young and Pellett 1994). Thus, when formulating

proteins from alternative sources, their EAA profiles and digestibility also need to be taken into consideration.

This review explores the protein concentration, the balance of EAA profiles and the gelling properties of proteins from a variety of sources: animals (meat, fish, crustaceans, eggs and milk), plants (cereals, legumes, nuts, roots, and other plants seeds which do not belong to the cereal or legume families), mushrooms, algae, and insects. The nutritional value of proteins from all alternative sources is compared to that of animal protein. The impact of processes as well as combinations of proteins and other macronutrient components on the final properties of the food are discussed in order to provide advice for food formulation. Standard and more innovative foods and dishes whose composition and processing can be optimized to obtain improved nutritional and sensory properties are presented.

How can protein nutritional value be assessed when formulating food

The nutritional value of proteins relies on quantity (protein concentration), quality (amino acid profile) and digestibility, as they all influence their final use by the body. To assess and compare the nutritional potential of animal and alternative proteins, emblematic food protein sources, either raw or processed (cooked, curdled, fermented), were selected in the scientific literature and in the USDA, CIQUAL and FEEDTABLES databases (Table 1).

Protein concentration of animal versus alternative sources

Protein concentration is crucial, since about 17% of energy intake is provided by protein (Afssa (Agence Française de Sécurité Sanitaire des Aliments) 2007, now called ANSES). The generally recognized protein intake recommendation (Afssa (Agence Française de Sécurité Sanitaire des Aliments) 2007) is based on the study of (Rand, Pellett, and Young 2003), a meta-analysis compiling 50 studies for a total of 830 subjects including intra-individual repetitions. The individual need was calculated with a linear interpolation of the data to obtain a neutral nitrogen balance and the average need corresponds to the median intake which satisfies the need of half the population. Considering a healthy adult subject, the average protein requirement was evaluated to 0.66 g/kg of body/day (Rand, Pellett, and Young 2003). Some authors proposed to use a 1.12 coefficient to calculate the intake recommendation in order to integrate the inter-studies, inter-individual and intra-individual variabilities which gave a recommended dietary allowance of 0.83 g/kg of body/day for proteins (Rand, Pellett, and Young 2003). Those different variabilities probably consider protein compositions in amino acids depending on their sources. Thus, the reference of 0.66 g/kg of body/day, was taken as the reference value for all the following calculations.

Protein concentrations are determined by conversion after direct measurement of the total concentration of nitrogen, with a conversion rate of 6.25. This factor (which dates

from the 19th century), is based on the hypothesis that food proteins contain 16% nitrogen, i.e., a 6.25 ratio. This principle, which is still widely accepted, does not stand up to a more detailed analysis (Mariotti, Tomé, and Mirand 2008). First, it has long been established that the proportion of nitrogen contained in proteins varies with the type of protein (Jones 1941). Those variations originate from the amino acid composition of proteins, some amino acids being richer in nitrogen than others. Second, the total nitrogen found in food sources is not only protein, or even amino acids, but includes other sources of non-protein nitrogen, such as nucleic acid, amine, urea, ammonia, nitrate, nitrite, phospholipids, etc. (Afssa (Agence Française de Sécurité Sanitaire des Aliments) 2007). In general, the values of the conversion factor differ slightly between animal and alternative protein sources, which is explained by the differences in amino acid composition and, above all, by the greater presence of non-protein nitrogen in certain products (Mariotti, Tomé, and Mirand 2008). In these cases, extrapolation is usually made from the meat conversion factor (6.25), leading to under- or over-estimate the protein content by up to 20%. However, in the case of insects, the 6.25 factor greatly overestimates the real protein concentration since the comestible chitinous shell provides additional non-protein nitrogen, and the protein content overestimation exceeds 30%. Consequently, some authors suggest the use of a corrected conversion factor value of 4.76 (Janssen et al. 2017), which is well below the usual 6.25 (by almost 24%). In this review, the conversion factor is the same as used in data sources, except for insects for which a corrected conversion factor of 4.76 is used. The protein concentration of raw materials from a wide variety of protein sources and some of the foods made from them are reported in Figure 1 and the corresponding conversion rates are detailed in Table 1.

The concentration of protein (in dry basis) from animal sources is 27% for milk, 53% for eggs, 37-83% for meats, and 58-90% for fish and crustaceans. The protein concentration of vegetal raw materials is well below that of meat and fish, 22-40% in legumes, 8-18% in cereals, 4-20% in nuts, 18-32% in other seeds and less than 10% in tubers. The concentration of protein in novel sources e.g., algae, mushrooms and insects is interesting, 16-66%, 20-30%, and about 37%, respectively. Food proteins can also be concentrated using industrial processes such as curdling and draining. For example, protein concentration increases from 27% in cow milk to 52% in mozzarella and from 27% in soymilk to 59% in soy tofu. Thus, if only based on the protein concentration, the best candidates to replace animal proteins would be legumes like soy or lupin, other seeds like pumpkin, white and brown mushrooms, insects like crickets and mealworms, and dried microalgae like spirulina or chlorella.

Amino acid composition of animal versus alternative sources

The nutritional value of a protein also depends on its amino acid balance with respect to human needs, in particular EAA (Afssa (Agence Française de Sécurité Sanitaire des Aliments) 2007; FAO (Food and Agriculture Organisation)/

Table 1. Data sources used to assess the nutritional potential of food proteins from animal and alternative sources.

Protein Source		Protein data		Amino acid data		N to Protein Conversion Factor
Source	State	Raw	Processed	Raw	Processed	Raw/Cooked
MEATS						
Beef	Lean meat	USDA 23094	USDA 23091	USDA 23094	USDA 23091	6.25
	Fat meat	USDA 13147	USDA 13148	USDA 13147	USDA 13148	[4]
Pork	Lean meat	USDA 10218	USDA 10222	USDA 10218	USDA 10222	6.25
	Fat meat	USDA 10088	USDA 10089	USDA 10088	USDA 10089	[4]
Chicken	Lean meat	USDA 5080	USDA 5082	USDA 5080	USDA 5082	6.25
	Fat meat	USDA 5075	USDA 5078	USDA 5075	USDA 5078	[4]
FISHES						
Tuna	Meat	USDA 15117	USDA 15118	USDA 15117	USDA 15118	[4]
Salmon	Meat	USDA 15236	USDA 15237	USDA 15236	USDA 15237	6.25
CRUSTACEANS						
Crab	Meat	USDA 15136	USDA 15137	USDA 15136	USDA 15137	[4]
Shrimp	Meat	USDA 15149	USDA 15151	USDA 15149	USDA 15151	6.25
EGGS AND MILK						
Milk	Liquid	USDA 1078	N/A	USDA 1078	N/A	[4]
	Yogurt	N/A	USDA 1116-	N/A	USDA 1116-	[4]
	Mozzarella	N/A	USDA 1028	N/A	USDA 1028	[4]
	Camembert	N/A	USDA 1007	N/A	USDA 1007	[4]
	Casein	N/A	N/A	Gordon et al. 1949	N/A	N/A
	Whey	N/A	N/A	USDA 1112	N/A	N/A
	Whole	USDA 1123	USDA 1131	USDA 1123	USDA 1131	6.25
	White, liquid	USDA 1124	N/A	USDA 1124	N/A	6.25
Egg	White, dried	N/A	USDA 1173	N/A	USDA 1173	6.25
	Yolk, liquid	USDA 1125	N/A	USDA 1125	N/A	6.25
	Yolk, dried	N/A	USDA 1137	N/A	USDA 1137	6.25
CEREALS						
Wheat	Seed	USDA 20076	USDA 56207200	USDA 20076	[2]	6.25
	Whole bread	N/A	USDA 18077	USDA 18077	N/A	5.95
	White bread	N/A	USDA 18416	USDA 18416	N/A	6.25
	Whole pasta	USDA 20124	USDA 20125	USDA 20124	USDA 20125	5.83
	White pasta	USDA 20420	USDA 20121	USDA 20420	USDA 20121	5.7
Rice	Gluten	N/A	N/A	(Woychik, Boundy, and Dimler 1961)	N/A	N/A
	Seed	USDA 20450	USDA 20451	USDA 20450	USDA 20451	5.8-5.9
	Seed	USDA 20014	USDA 11168	USDA 20014	USDA 11168	6.25
	Oats	Seed	USDA 20038	[3]	USDA 20038	6.38
	Barley	Seed	USDA 20005	USDA 20006	USDA 20005	6.25
	Rye	Seed	USDA 20062	[3]	USDA 20062	5.83
Spelt	Seed	USDA 20140	USDA 20141	USDA 20140	USDA 20141	5.83-6.25
LEGUMES						
Soy	Seed	USDA 16108	USDA 16109	USDA 16108	USDA 16109	[4]
	Milk	N/A	USDA 16325	N/A	[1]	6.25
	Yogurt	N/A	USDA 43476	N/A	[1]	[4]
	Tofu	N/A	USDA 16162	N/A	USDA 16162	[4]
	Isolate	N/A	N/A	USDA 16122	N/A	N/A
	Seed	USDA 16085	USDA 16086	USDA 16085	USDA 16086	6.25
Split pea	Seed	USDA 16056	USDA 16057	USDA 16056	USDA 16057	6.25
Chickpea	Seed	USDA 16069	USDA 16070	USDA 16069	USDA 16070	6.25
Green lentil	Seed	USDA 16032	USDA 16033	USDA 16032	USDA 16033	[4]
Red bean	Seed	USDA 16076	USDA 16077	USDA 16076	USDA 16077	[4]
Lupin	Seed	USDA 16087	USDA 16088	USDA 16087	USDA 16088	5.46
Peanut	Seed	USDA 11354	USDA 11357	USDA 11354	USDA 11357	6.25
TUBERS						
French potato	Root	USDA 11507	USDA 11508	USDA 11507	USDA 11508	6.25
Sweet potato	Root	USDA 11134	USDA 787178	USDA 11134	USDA 787178	6.25
Cassava	Root	USDA 11601	USDA 11602	USDA 11601	USDA 11602	[4]
Yam	Root	USDA 11518	USDA 11519	USDA 11518	USDA 11519	[4]
NUTS						
Cashew	Nut	USDA 12087	[3]	USDA 12087	[3]	5.3
Almond	Nut	USDA 12061	[3]	USDA 12061	[3]	5.18
Chestnut	Nut	USDA 12098	[3]	USDA 12098	[3]	[4]
Walnut	Nut	USDA 12155	[3]	USDA 12155	[3]	5.3
Hazelnut	Nut	USDA 12120	[3]	USDA 12120	[3]	5.3
SEEDS						
Quinoa	Seed	USDA 20035	USDA 20137	USDA 20035	USDA 20137	6.25
Sesame	Seed	USDA 12023	[3]	USDA 12023	[3]	[4]
Flax	Seed	USDA 12220	[3]	USDA 12220	[3]	5.3
Chia	Seed	USDA 12006	[3]	USDA 12006	[3]	5.3
Pumpkin	Seed	USDA 12014	[3]	USDA 12014	[3]	5.3
Sunflower	Seed	USDA 12036	[3]	USDA 12036	[3]	5.3
Rapeseed	Seed	Feed tables [6]	[3]	Feed tables [6]	[3]	[4]
MUSHROOMS						

(continued)

Table 1. Continued.

Protein Source	Source	Protein data		Amino acid data		N to Protein Conversion Factor Raw/Cooked
		State	Raw	Processed	Raw	
O. Mushroom	Whole		USDA 11987	USDA 11264	USDA 11987	USDA 11264
<i>P. osteratus</i>						6.25
W. mushroom	Whole		USDA 11260	USDA 11261	USDA 11260	USDA 11261
<i>A. bisporus</i>						6.25
Br. mushroom	Whole		USDA 11265	USDA 11243	USDA 11265	USDA 11243
<i>A. brunnescens</i>						6.25
S. mushroom	Whole		USDA 11238	USDA 11269	USDA 11238	USDA 11269
<i>L. edodes</i>						6.25
M. mushroom	Whole		USDA 11993	[1]	USDA 11993	[1]
<i>G. frondosa</i>						6.25
E. mushroom	Whole		USDA 11950	[1]	USDA 11950	[1]
<i>F. velutipes</i>						6.25
ALGAE AND BACTERIA						
Spirulina	Dried		Becker 2004, 2007	[3]	Becker 2004, 2007	[3]
<i>A. platensis</i>						6.25
Spirulina	Dried		Becker 2004, 2007	[3]	Becker 2004, 2007	[3]
<i>A. maxima</i>						6.25
Chlorella	Dried		Becker 2004, 2007	[3]	Becker 2004, 2007	[3]
<i>C. vulgaris</i>						6.25
Wakame	Fresh		USDA 11669	[3]	USDA 11669	[3]
<i>U. pinnatifida</i>						[4]
Nori	Dried		Dawczynski, Schubert, and Jahreis 2007	[3]	Dawczynski, Schubert, and Jahreis 2007	[3]
<i>P. tenera</i>	Dried		Fleurence 1999	[3]	Fleurence 1999	[3]
INSECTS						
Yell. mealworm	Fresh		Finke 2013 Yi et al. 2013 Azagoh et al. 2016 *****	[3]	Finke 2013 Yi et al. 2013 Azagoh et al. 2016 *****	[3]
						4.76 [5]
House cricket	Fresh					4.76 [5]
<i>A. domesticus</i>						

N/A: Not appropriate for the considered product in the considered state. [1] Some products with missing data were excluded from certain parts of the study. [2] Processed products with missing data were replaced using data on raw whole products. [3] Processed products with missing data were only considered raw. [4] The nitrogen-to-protein conversion factor was not indicated and was assumed to be 6.25. [5] The protein concentrations from insects have been corrected with a conversion factor of 4.76 instead of 6.25. [6] Feedinamics is available at: <https://feedtables.com/fr/content/graine-de-colza>.

WHO (World Health Organisation)/UNU (United Nations University) 2007). Twenty amino acids are required by the human metabolism, including 9 non-essential and 11 essential amino acids (EAA are listed in Table 2). EAA cannot be synthesized and can only be provided by food. Protein metabolism requires a balance between the EAA provided in specific amounts. If there is a deficiency in one or more EAA, the body protein synthesis rate slows down to the most limiting EAA. In this case, excess amino acids, whether essential or not, are degraded and lost in the urine (Chardigny and Walrand 2016). The “ideal” daily amino acid requirement is expressed as a reference protein, *i.e.*, which meets the need for each essential amino acid (Table 2). It was calculated by dividing the daily need for each EAA (in mg/kg of body/day) by the daily protein need of a healthy adult (0.66 g/kg of body/day) (Table 2).

Presentation of usual indicators to assess protein quality

Two nutritional indicators are often used to compare the essential amino acid profiles of food proteins (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007). The first, called “Essential Amino Acid Index” (EAAI), makes it

possible to determine whether the protein meets the human need for each EAA (Equation 1). The EAAI_(i) is calculated for each essential amino acid (i) among the 11 EAA of a given protein. It is the ratio of the AA content in the protein to the content of the same EAA in the reference protein. Compared to the reference protein for which all EAAI_(i) = 1, if EAAI_(i) < 1 the amino acid (i) is insufficient, if EAAI_(i) > 1 the amino acid (i) is in “excess” vs the minimum human need.

$$\text{EAAI}_{(i)} = \frac{(\text{EAA}_{(i)} \text{ of the protein})}{(\text{EAA}_{(i)} \text{ of the reference})} \text{ for each of the 11 EAA of the protein} \quad (1)$$

The second indicator, called “Limiting Amino Acid Index” (LAAI), refers to the lowest EAAI_(i) of all essential amino acids (i) of a protein (Equation 2). Compared to the reference protein for which LAAI = 1, if LAAI < 1 the protein does not meet the needs (meaning limited protein synthesis), if LAAI ≥ 1 the protein meets the needs.

$$\text{LAAI} = \min_{(i)=1 \text{ to } 11}^{\text{EAAI}_{(i)}} \text{ among the 11 EAAI of the protein} \quad (2)$$

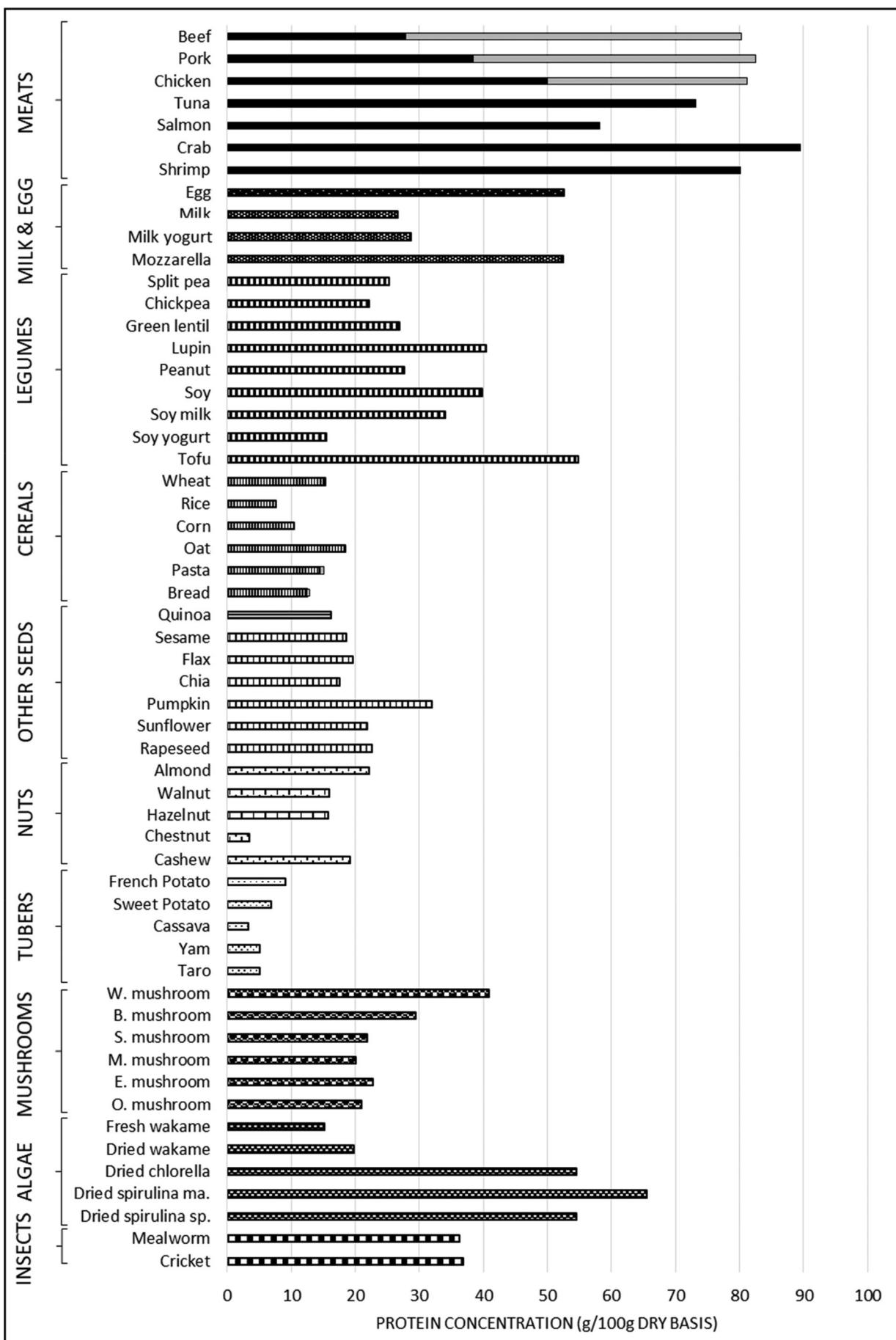


Figure 1. The concentration of protein from animal and alternative sources. The light gray bars give an idea of the distance between the minimum and the maximum values found in the different sources. Data sources are listed in Table 1.

Table 2. Amino acid needs for human metabolism in adults over 18 years old and amino acid profile of Reference Protein (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007).

	TRP	TRH	ISO	LEU	LYS	MET	CYS	VAL	HIST	PHE + TYR
Amino acid needs for human metabolism (mg AAI· Kg ⁻¹ , J ⁻¹)	4	15	20	39	30	-	-	26	10	25
Amino acid profile of the Reference Protein (mg AAI· g Protein ⁻¹)	6	23	30	59	45	16	6	39	15	38

New indicators proposal to allow protein comparison

Due to the importance of the limiting amino acid, two new indexes are proposed here: one that quantifies the extent to which each EAA_(i) is in excess compared to the limiting amino acid and the other that evaluates the global loss of EAA if the corresponding protein is eaten alone. The Standardized Amino Acid Index (SAAI_(i)) was calculated for each essential amino acid (i) among the 11 EAA of a given protein (Equation 3). Compared to the reference protein for which all SAAI_(i) = 1, the higher SAAI_(i) above 1, the higher the excess EAA.

$$\text{SAAI}_{(i)} = \frac{\text{EAAI}_{(i)}}{\text{LAAI}} \text{ for each of the 11 EAA of the protein} \quad (3)$$

The Loss Index (LI) quantifies the imbalance between the limiting essential amino acid and the other EAAs (Equation 4). LI refers to the sum of the differences between the SAAI_(i) of all EAA(i) and the SAAI of the limiting essential amino acid (=1) for a given protein. A high LI value means a high imbalance between the limiting EAA and all the others, and thus a high theoretical loss of amino acids.

$$\text{LI} = \sum_{i=1}^{11} (\text{SAAI}_{(i)} - 1) \quad (4)$$

Even if the 4 indicators described above (Equations 1 to 4) are primarily based on a chemical analysis and do not consider the bioavailability of EAA, they are suitable for screening and categorizing protein sources.

Assessment of proteins from different sources as regards both indicators

Examples of EAAI and SAAI profiles are given in Figure 2 for main protein sources. In the left part of Figure 2 (A1 to J1), which shows the EAAI, light gray area corresponds to the EAAI of the reference protein, while in the right part of Figure 2 (A2 to J2), which shows the SAAI, light gray area corresponds to the LAAI of the considered protein. The EAAI representations make it easy to see which EAAs_(i) are within the light gray area, *i.e.* limiting with respect to the reference protein, and which ones are outside the light gray area, *i.e.* in excess with respect to the reference protein. One can see if the EAA profile is balanced with respect to the reference protein. The SAAI_(i) representation gives an overview of the balance between the limiting EAA and the excess EAAs_(i) showing the level of potential loss of each EAA_(i) due to an imbalance with the most limiting EAA_(i). The bigger the area between the light gray area and the protein curve, the higher the potential total loss of EAA.

Not surprisingly, the fact that all the curves in Figure 2-A1 are clearly outside the light gray area corresponding to the reference protein, confirms there is no limiting EAA in animal sources (meat, milk, eggs, sea products). However, three groups can be distinguished: meat from mammals, fish and crustaceans, milk, and egg proteins. The difference between the three groups mainly concerns cysteine, in huge excess in eggs (see EAAI and SAAI, Figure 2-A1 and -A2), histidine and lysine in excess in meats. Milk has the most balanced profile close to the gray circle in SAAI and the lowest LI (2.4). Whatever their origin, the loss index (LI) of animal proteins nevertheless remained the smallest of proteins from all origins, with values ranging from 2 to 4.

Proteins from legumes and cereals also constitute two very homogenous groups with very similar EAAI profiles. However, in contrast to legume proteins, cereal proteins are limited in lysine and balanced in methionine. In all legume and cereal proteins (Figure 2-B1 and -C1), we observed a notable excess of cysteine, aromatic AA, and to a lesser extent, of tryptophan. Compared to animal proteins, the SAAI of cereal and legumes varies markedly in surface area between the light gray center and the curves and in the level of excess EAA (Figure 2-B2 and -C2). Among legumes, soy has the smallest surface area corresponding to a LI of 7, and lupin has the biggest, with an LI of 20. Among cereals, oat has the smallest surface area corresponding to a LI of 8, and wheat the biggest with an LI of 17. Thus, in all cases, the LI values calculated for cereals and legumes are much higher than for animal proteins.

Tuber proteins are limited in either leucine, lysine or methionine (Figure 2-E1). Interestingly, proteins from cashew and chestnut have no limiting amino acids compared to the reference protein (Figure 2-F1). Other nut proteins are limited in lysine and methionine. Among seeds, proteins from quinoa, flax, chia, sunflower and rapeseed have no limiting amino acid compared to the reference protein (Figure 2-G1). Sesame and pumpkin are only limited in lysine. Most tuber, nut and seed proteins have at least a noticeable excess of cysteine and tryptophan (Figure 2-E1, -F1, and -G1). In terms of SAAI, among tubers potato, among nuts, cashew and chestnut, and among seeds, quinoa have the smallest surface areas and hence the smallest LI values (Figure 2-E2, -F2, and -G2). With values of 4-5, they are not so far from the LI range found for animal proteins.

All proteins of vegetal origin have higher loss indexes (LI) than proteins from animal sources. Distinct ranges with three different levels can be defined: medium (5-10), high (10-15) and very high (15-20) levels compared to the low LI of animal proteins. Proteins from tubers (LI from 5 to 9) form homogeneous groups with medium LI while proteins from cereals (LI from 8 to 17), legumes (LI from 7 to 20),

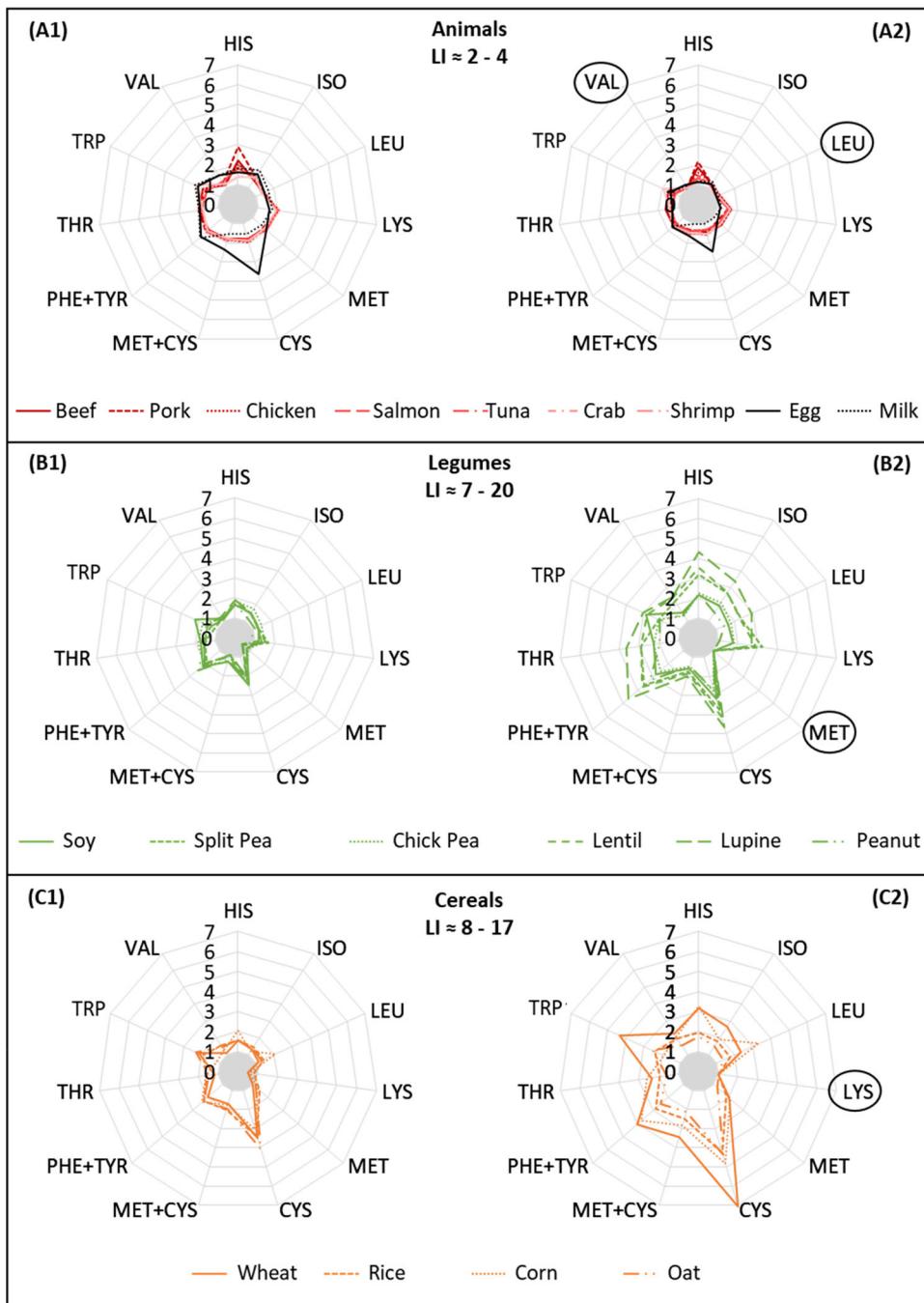
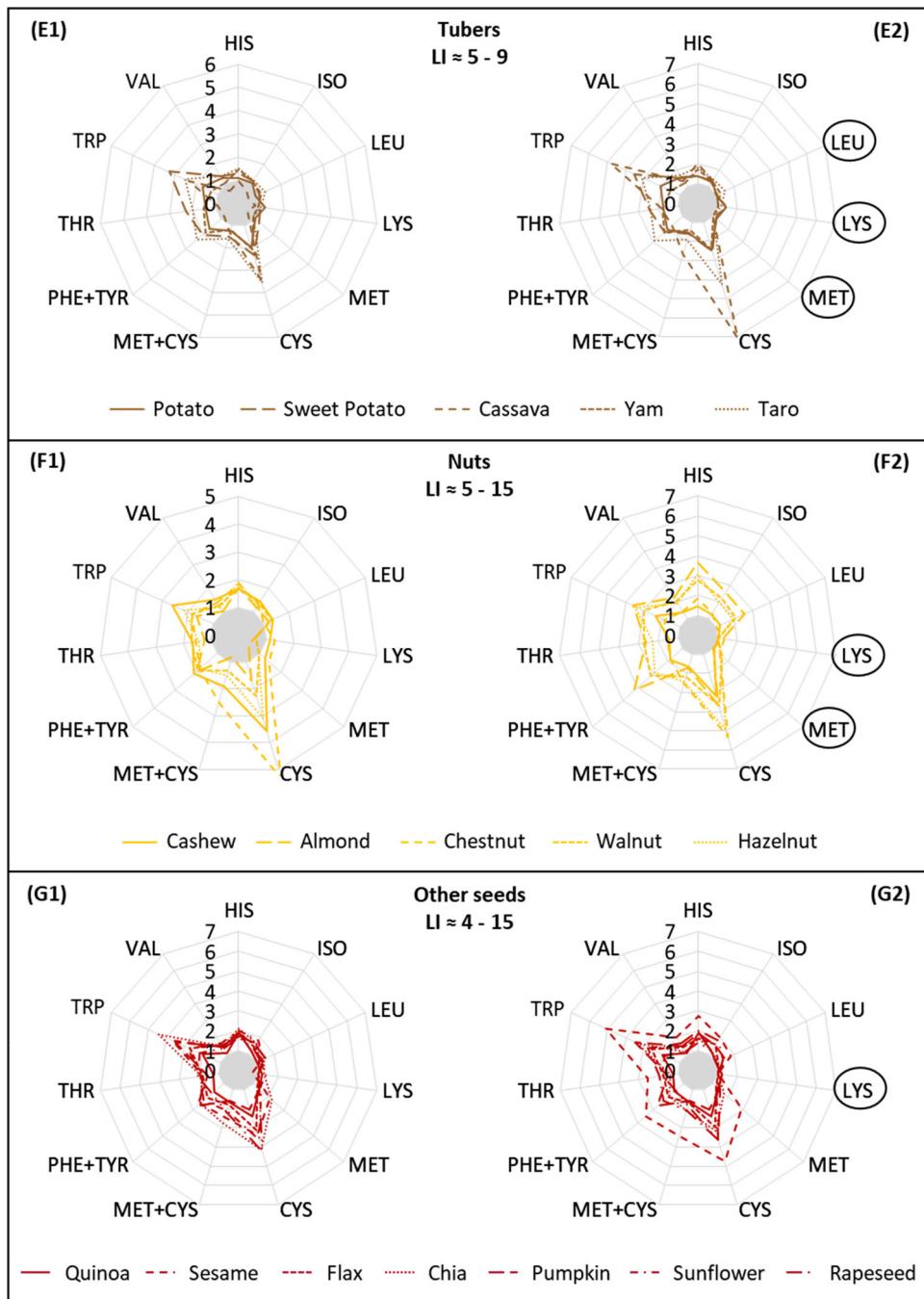


Figure 2. Essential amino acid indexes EAAI (left, A1 to J1) and standardized amino acid indexes SAAI (right, A2 to J2) of food proteins from different sources calculated from the data listed in Table 1, using Equation 1 and Equation 3, respectively. In the left part of the Figure 2 (A1 to J1) showing the EAAI, light gray area corresponds to the EAAI of the reference protein, while in the right part of the Figure 2 (A2 to J2) showing the SAAI, light gray area corresponds to the LAAI of the considered protein. The limiting essential amino acids are identified by black circles and the Loss Index (LI) ranges are given for each protein group.

nuts (LI from 5 to 15) and seeds (LI from 4 to 15) are much more heterogeneous. It should be noted that some species (oats, rice, peanut, rapeseed, cashew, chestnut, and quinoa) have a medium LI, and are much more balanced than others (wheat, lentil, lupin, almond, sesame) which have a high LI. Thus, the balance between amino acids appears to be highly dependent on the botanical variety.

Insects, fungi, and algae form very different groups in terms of profile. Although insects appear to be very balanced in Figure 2-H1, the lack of methionine in their protein makes them less balanced than animal sources. Yellow

mealworms have a larger SAAI surface area (Figure 2-H2) corresponding to an LI in the high range, whereas lesser mealworms and crickets have a smaller SAAI surface area with a corresponding LI of 7, i.e. in the medium range. Mushrooms, bacteria, and algae form heterogeneous groups. All mushroom proteins are limited in two to six EAA, meaning they are of poor nutritional quality. Proteins from algae form the most heterogeneous group with big differences ranging from microalgae (no limiting EAA for chlorella) to macroalgae (methionine insufficiency in wakame) but also within the same genus of microalgae such as *Spirulina*

**Figure 2.** Continued.

platensis and *Spirulina maxima*. The LI of proteins from insects, algae, mushrooms and bacteria vary with the species: from a low level (4 for chlorella) to an extremely high level (28 for wakame).

In terms of amino acid balance and the resulting low or medium LI, several candidates from different alternative sources can be used to replace animal proteins: cashew or chestnut (nuts); quinoa, flax, chia, pumpkin or rapeseed (other seeds); chlorella (microalgae); oyster mushrooms; cricket or lesser mealworms (insects); soy, chickpea, peanut (legumes); potato and yam (tubers). Taking the protein concentration of such protein sources into account (Figure 1, section 2.1), the best ones on this first list would be

pumpkin, chlorella, oyster mushrooms, crickets, mealworms, and soy.

However, it should also be noted that food processing can also affect the concentration and composition of protein. Previous studies (Domínguez, Borrajo, and Lorenzo 2015; Mitchell, Hamilton, and Beadles 1949; Wu et al. 1994) reported significant variations in the concentration in meat and plant amino acids depending on the cooking conditions. Thermal variations and the addition of non-protein ingredients such as oils or sugars during cooking, cause oxidation, reduction reactions, conversion and release of free amino acids (Domínguez, Borrajo, and Lorenzo 2015). These reactions can cause major changes in the proportions

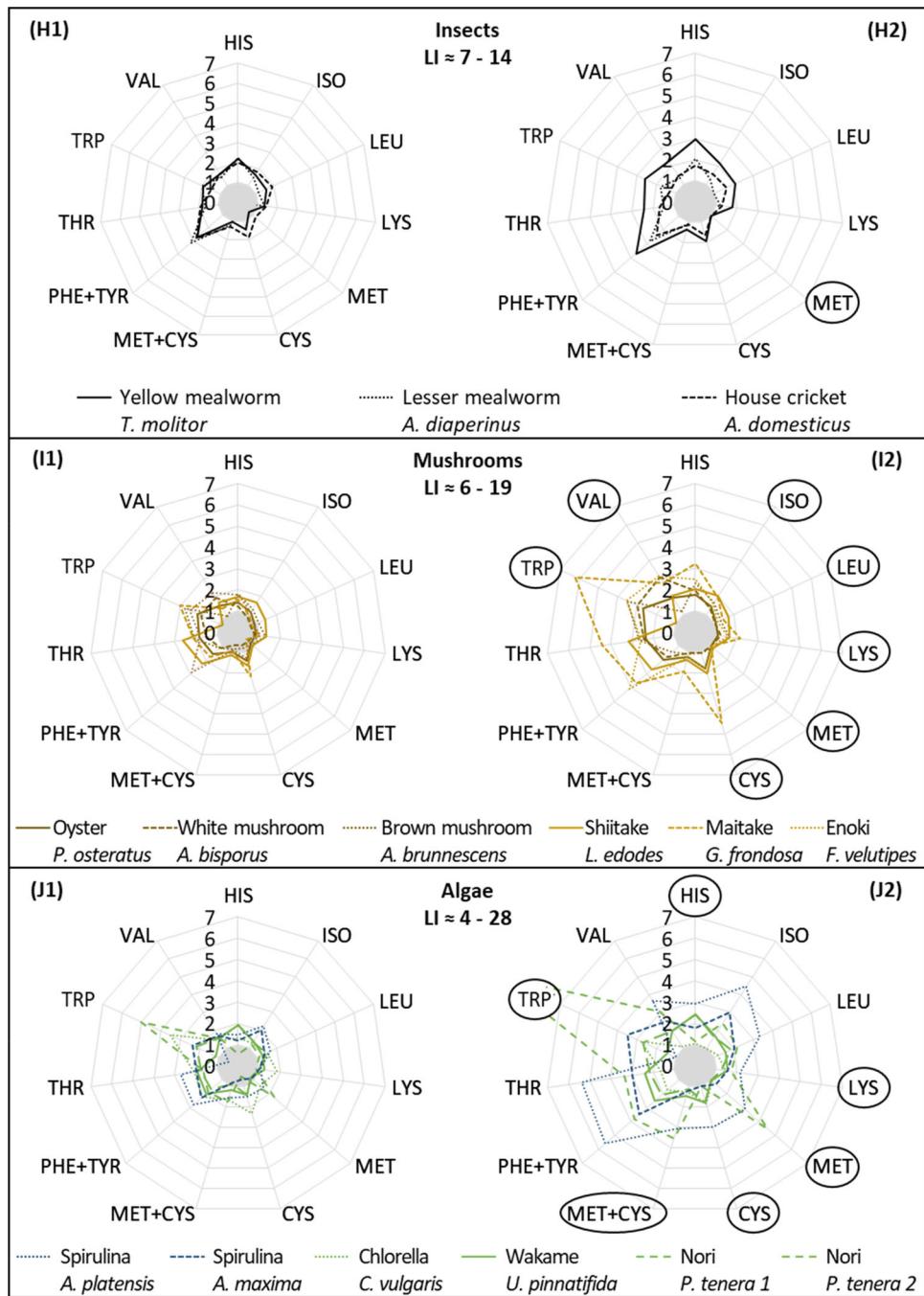


Figure 2. Continued.

of essential amino acids and hence in the EAA profiles. For a given protein, the quantity or type of limiting amino acids may even change to another of the 10 other EAA. The result may be an improvement (meats) or a degradation (grains) of the profile balance and of the LI. (Steinkraus 1994) reported that the concentration of lysine in rice and the concentration of methionine in legumes increased considerably with Indonesian “Tapai” fermentation and Indian “Idli” fermentation processes, while the essential amino acid profile degraded when raw milk was transformed into mozzarella or camembert. It seems that biochemical treatments (like rennet) and microbial treatments (with ferments) may also either degrade or improve the EAA profile of protein

sources in raw food. Finally, in most vegetal species, the EAA profile of the whole seed differs from that of the flour, the bran or the germ (Lászity and Hidvégi 1983). Indeed, since the proteins in the different parts of the seeds are not the same, refining processes such as hull or germ removal obviously affect the essential amino acid profile. Thus, for food formulation, it is more appropriate to note the amino acid profiles of the final products, either refined, cooked, or fermented. It should also be noted that the chemical transformations of amino acids during the processing and preparation of food could modify their digestibility, their bioavailability and, ultimately, their biological fate. One of the main reactions that occurs when food proteins are

Table 3. Protein digestibility and evaluation method, limiting essential amino acid (LEAA), recalculated limiting essential amino acid index (LAAI), loss index (LI), for foods from different origins in different states.

	Protein source nature	Protein state	LEAA	LAAI (Eq.2)	LI (Eq.4)	Protein digestibility (%)	Digestibility evaluation method	Digestibility bibliographic references	
ANIMAL PRODUCTS	Beef	cooked	VAL	1.23	3.4	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Pork	cooked	LEU	1.37	3.4	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Chicken	cooked	VAL	1.25	3.9	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Salmon	cooked	LEU	1.32	2.9	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Tuna	cooked	VAL	1.32	2.9	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Crab	cooked	VAL	1.21	3.7	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Shrimp	cooked	VAL	1.20	3.8	90 - 99	<i>in-vivo</i> (human)	[11] [12] [29] [34]	
	Egg	raw	LEU	1.47	2.7	51	<i>in-vivo</i> (human)	[10]	
		cooked	LEU	1.47	2.7	91 - 100	<i>in-vivo</i> (human)	[11] [12] [29] [34] [39]	
		ovalbumin	LEU	1.46	3.3	99	<i>in-vivo</i> (human)	[29]	
CEREALS	Wheat*	Milk	liquid	CYS	1.52	2.4	90 - 100	<i>in-vivo</i> (human)	[12] [13] [29] [34]
		yogurt	TRY	0.96	8.2	90 - 100	<i>in-vivo</i> (human)	[11] [12] [39]	
		mozzarella	CYS	0.99	9.2	92 - 98	<i>in-vivo</i> (human)	[11] [12] [39]	
		camembert	CYS	0.92	10.4	92 - 98	<i>in-vivo</i> (human)	[11] [12] [39]	
		caseins	CYS	0.57	22.5	97-100	<i>in-vivo</i> (human)	[12] [29]	
		globulins	TRY	1.15	5.5	83-94	<i>in-vivo</i> (human)	[6]	
		whole flour	LYS	0.60*	12.2*	77 - 93	<i>in-vivo</i> (human)	[11] [12] [29] [34] [39]	
		white flour	LYS	0.49	16.4*	92 - 96	<i>in-vivo</i> (human)	[11] [12] [39]	
		bread	LYS	0.65	11.8	85 - 99	<i>in-vivo</i> (human)	[26]	
		gluten	LYS	0.00	36.3	96 - 99	<i>in-vivo</i> (human)	[11] [12] [39]	
LEGUMES	Soy	Rice	cooked seed	LYS	0.80	9.2	75 - 88	<i>in-vivo</i> (human)	[11] [12] [39]
		Corn	cooked seed	LYS	0.92	5.7	70 - 87	<i>in-vivo</i> (human)	[11] [12] [29] [39]
		Soy	cooked seed	MET	0.77	10.0	78	<i>in-vivo</i> (human)	[11] [12] [29] [34] [39]
			cooked flour	MET	0.77	10.0	70 - 93	<i>in-vivo</i> (human)	[11] [12] [29] [34] [39]
			isolate	MET	0.80	8.8	93 - 98	<i>in-vivo</i> (human)	[11] [12] [29] [34] [39]
		Green pea	cooked seed	MET	0.64	12.3	83 - 84	<i>in-vivo</i> (human)	[12]
		Chickpea	cooked seed	MET	0.82	7.5	88 - 89	<i>in-vivo</i> (human)	[12]
		Lentil	cooked seed	MET	0.53	16.1	84 - 85	<i>in-vivo</i> (human)	[12]
		Peanut	raw seed	MET	0.77	7.3	96	<i>in-vivo</i> (human)	[29]
			raw butter	-	-	-	92 - 98	<i>in-vivo</i> (rat, human)	[29] [31]
OTHER SEEDS	Quinoa		raw meal	-	-	91	<i>in-vivo</i> (rats)	[29]	
			raw flour	-	-	90 - 95	<i>in-vivo</i> (rats)	[29] [4]	
			cooked meal	-	-	96	<i>in-vivo</i> (rats)	[30]	
		Flax	cooked seeds	LEU	1.01	3.8	83 - 85	<i>in-vitro</i> (enzymes)	[28]
			raw seeds	LYS	0.71	15.1	90	<i>in-vitro</i> (enzymes)	[15]
			raw seeds	LYS	1.05	5.7	13	<i>in-vitro</i> (enzymes)	[23]
			degum. flour	-	-	51	<i>in-vitro</i> (enzymes)	[23]	
			degum. defat. flour	-	-	67	<i>in-vitro</i> (enzymes)	[23]	
			toasted flour	-	-	28 - 32	<i>in-vitro</i> (enzymes)	[23]	
			isolate	-	-	68	<i>in-vitro</i> (enzymes)	[23]	
NUTS	Chia		raw seeds	LYS	1.30	5.9	29	<i>in-vitro</i> (enzymes)	[24]
			toasted seeds	-	-	11	<i>in-vitro</i> (enzymes)	[24]	
			soaked seeds	-	-	24	<i>in-vitro</i> (enzymes)	[24]	
			raw flour	-	-	80	<i>in-vitro</i> (enzymes)	[24]	
			toasted flour	-	-	34	<i>in-vitro</i> (enzymes)	[24]	
			isolate	-	-	49	<i>in-vitro</i> (enzymes)	[24]	
		Rapeseed	raw whole seed	LEU	1.17	4.0	19	<i>in-vivo</i> (human)	[35]
			raw ground seed	-	-	74 - 81	<i>in-vivo</i> (pig)	[3] [33] [35]	
			raw dehul. meal	-	-	86	<i>in-vivo</i> (pig)	[3]	
			raw dehul. defat. meal	-	-	87	<i>in-vivo</i> (pig)	[3] [36] [32]	
TUBERS	Sunflower		heat. dehul. defat. meal	-	-	74	<i>in-vivo</i> (pig)	[32]	
			raw defat. kernel meal	-	-	80	<i>in-vivo</i> (pig)	[32] [35]	
			isolate	-	-	84 - 95	<i>in-vivo</i> (human, rat)	[5] [27] [38]	
			hydrolysate	-	-	97	<i>in-vivo</i> (rat)	[38]	
			raw whole meal	LYS	1.00	7.9	90 - 91	<i>in-vivo</i> (rat)	[12] [39]
			heated whole meal	-	-	90	<i>in-vivo</i> (rat)	[29]	
			raw dehul. defat. meal	-	-	93 - 96	<i>in-vivo</i> (rat)	[7] [25]	
			raw degum. defat. meal	-	-	78 - 79	<i>in-vivo</i> (rats)	[14]	
			raw, meal	LYS	0.91	7.7	88	<i>in-vitro</i> (enzymes)	[22]
			raw seed	MET	0.46	15.5	83 - 92	<i>in-vitro</i> (enzymes)	[1]
MUSHROOMS	ALGAE & BACTER.	Potato	raw seed	LYS	1.13	4.6	84 - 92	<i>in-vitro</i> (enzymes)	[16] [17]
		Pleurotus	cooked, whole	LEU	0.81	5.1	73	<i>in-vitro</i> (enzymes)	[18]
		Agaricus	cooked, whole	LEU	0.62	7.6	73	<i>in-vivo</i> (rats)	[9]
		Spirulina (Spirulina)	dried, whole	CYS	0.67	13.1	74 - 84	<i>in-vivo</i> (rats)	[2] [6]
		Chlorella	dried, whole	ISO	1.27	4.3	54 - 89	<i>in-vivo</i> (rats)	[2] [21]
		Undaria (Wakame)	dried, whole	MET	0.80	8.0	86	<i>in-vivo</i> (rats)	[37]
		Molitor	raw, meal	MET	0.62	14.1	90 - 93	<i>in-vivo</i> (pigs)	[20] [41]
			supernatant	-	-	75 - 85	<i>in-vitro</i> (enzymes)	[40]	
			pellet	-	-	29 - 50	<i>in-vitro</i> (enzymes)	[40]	
			residue	-	-	13 - 24	<i>in-vitro</i> (enzymes)	[40]	

*Wheat LAAI and LI are those of the raw seed/flour since amino acid content was not available for the cooked seed/flours. Eq: Equation. Heat heated. Degum: degummed. Dehul: dehulled. Defat.: defatted. Bacte.: bacteria. The table is adapted from the following sources. [1] (Ahrens et al. 2005) [2] (Becker 2007) [3] (Bell 1993) [4] (Bodwell, Satterlee, and Hackler 1980) [5] (Bos et al. 2007) [6] (Calvez et al. 2019) [7] (Canibe et al. 1999) [8] (Clement, Giddey, and Menzi 1967) [9] (Dabbour and Takruri 2002) [10] (Evenepoel et al. 1998) [11] (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 1985) [12] (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation) 1991) [13] (FAO (Food and Agriculture Organisation) 2011) [14] (FAO (Food and Agriculture Organisation) 2012a) [15] (Fasuan, Gbadamosi, and Omobuwajo 2018) [16] (Fetuga, Babatunde, and Oyenuga 1974) [17] (Freitas et al. 2012) [18] (Gahlawat and Sehgal 1998) [19] (Goyal, Grewal, and Goyal 2006) [20] (Jin et al. 2016) [21] (Lubitz 1963) [22] (Mansour et al. 1993) [23] (Marambe, Shand, and Wanasyundara 2013) [24] (Monroy-Torres et al. 2008) [25] (Mitchell, Hamilton, and Beadles 1949) [26] (Murlin, Marshall, and Kochakian 1941) [27] (Rozan et al. 1997) [28] (Ruales and Nair 1992) [29] (Sarwar 1987) [30] (Sarwar and Peace 1986) [31] (Sarwar et al. 1989) [32] (Sarwar, Shannon, and Bowland 1975) [33] (Sauer, Cichon, and Misir 1982) [34] (Reeds et al. 2000) [35] (Skiba et al. 1999) [36] (Thompson and Serraino 1986) [37] (Urbano and Goñi 2002) [38] (Wanasundara et al. 2016) [39] (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007) [40] (Yi et al. 2016) [41] (Yoo et al. 2019).

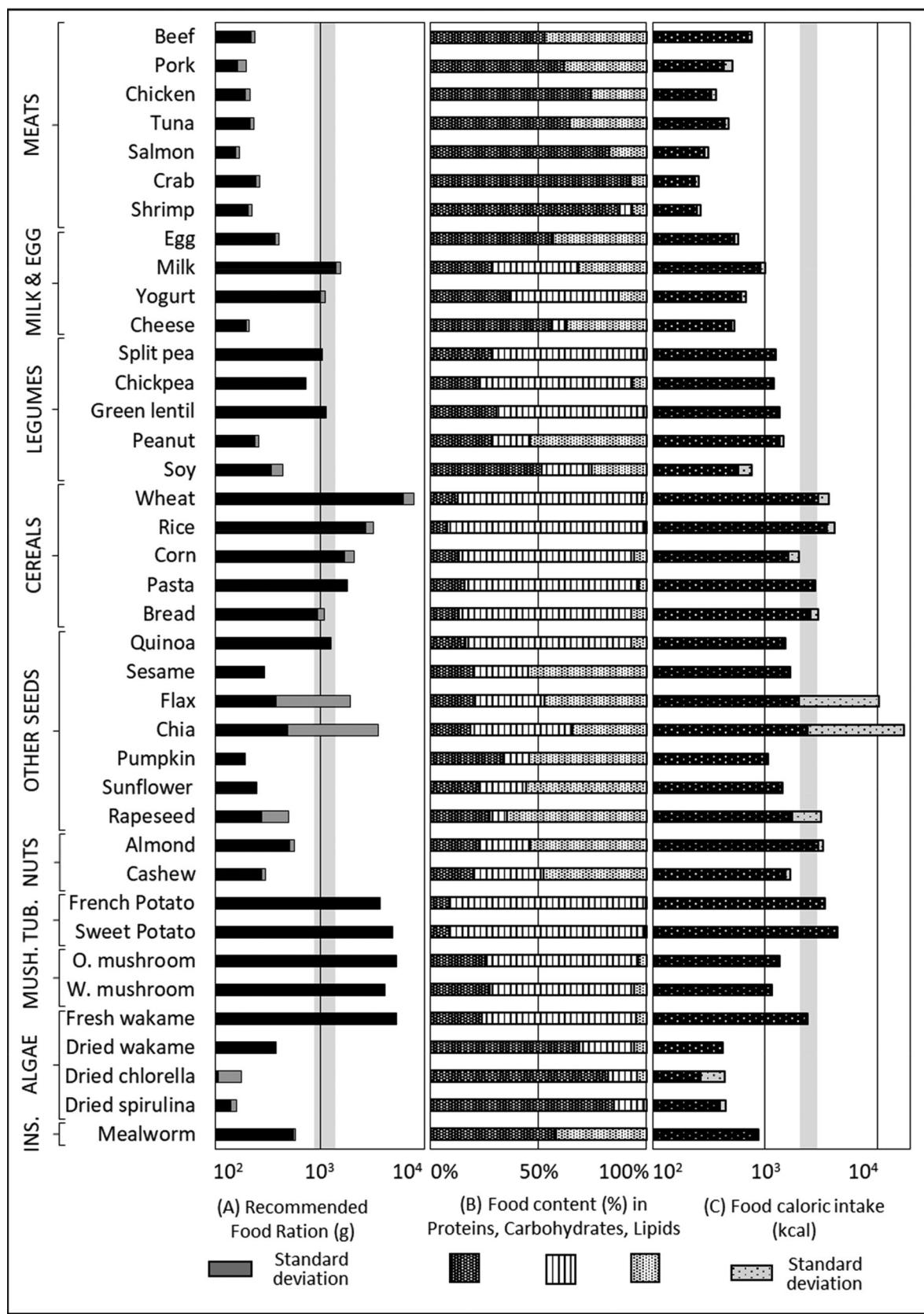


Figure 3. Animal and alternative sources of protein evaluation in terms of (A) Recommended Food Intake (RFI) needed individually to meet the nitrogen and amino acid needs, (B) macronutrient composition, (C) corresponding caloric intake (CI). The light gray vertical bands delimit on Figure 3-A the reasonable daily ration suggested by (Rolls, Morris, and Roe 2002) and on Figure 3-C the caloric intake as defined by (EFSA (European Food Safety Authority) 2016). Processed flax integrates the digestibility of both degummed and defatted flax.

heated, is the Maillard reaction, which occurs between free amine and carbonyl groups within food systems. Although there are many beneficial effects of the Maillard reaction in foods, such as improved flavor, the reaction can also have detrimental effects, primarily nutritional. One of the possible adverse effects of the Maillard reaction is reducing the nutritional quality of the protein by reducing the availability of certain amino acids, particularly lysine. On the other hand, if dietary intake of protein is high and if sufficient quantities of the EAA are consumed, the reduction in protein quality due to Maillard-type reactions may be have no consequences. It should however be noted that people on restricted diets, e.g., older people, may be vulnerable to reduced protein intake given the limited variety of foods they are likely to consume.

Digestibility of proteins from animal versus alternative sources

Protein nutritional value also depends on protein digestibility *i.e.* or on the release of small peptides ready for intestinal absorption (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007). The different methods of determining protein digestibility, either *in vivo* or *in vitro*, either apparent or real, fecal or ileal, with variable enzymes or using animals or humans, have been widely defined and discussed (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation) 1991; FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 1985, FAO (Food and Agriculture Organisation) 2011, 2012a; FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007).

Protein digestibility values and the limiting EAAs of protein sources are listed in Table 3. The limiting EAAs of processed material differ from those of the protein in the raw material. LAAI and LI values were recalculated using the EAA profiles of processed proteins and are reported in Table 3. Digestibility values of cooked meats, eggs, cereals, legumes, tubers can be found in the literature but digestibility values of the raw form of nuts, oilseeds and raw insects were used since no data were found on their heat-treated form. The digestibility data were obtained using different digestion methods and differed among species, varieties, cultivars, growing and processing conditions (Bressani and Elias 1977). Thus, these values are approximations but, nevertheless, adequately illustrate the wide range of protein digestibility among the different sources, and the effects of processing.

Animal proteins are more digestible than most proteins from non-animal sources (Table 3). In vegetal proteins, two mechanisms reduce digestibility. On the one hand, cell walls or tangled macromolecules (mucilaginous carbohydrate, cellulose, pectin, or xylan) may act as physical barriers (Liu et al. 2018; Marambe, Shand, and Wanasaundara 2013; Schneeman 1978). Hence, large intakes of bran and other fiber-rich foods may increase the excretion of nitrogen in

the feces and reduce apparent protein digestibility by about 10% (FAO (Food and Agriculture Organisation) 1985). Oils also interfere with enzymatic activity through formation of liposomes, emulsions, or micelles, removing amphiphilic proteins, which move to the oil/water interfaces thereby becoming less available. On the other hand, chemical components act as anti-nutritional factors, *i.e.* vegetal compounds able to inhibit protein digestion. For example, vegetal cells contain hemagglutinins, tannins, lectins, saponins, polyphenols, phytic acid or trypsin inhibitors which inhibit enzymatic proteolysis (Khalil and Mansour 1995; Rehman and Shah 2005; Wu et al. 1994).

Protein digestibility is still highly sensitive to physical conditions and can be either improved or degraded by food production and processing (FAO (Food and Agriculture Organisation) 2011). Indeed, processing may affect protein conformation through denaturation and/or aggregation but also accessibility through the resulting composition and microstructures that surround it. Heat treatment may destroy some anti-nutritional factors (Khalil and Mansour 1995). Significant improvements have been reported for legumes (Rehman and Shah 2005), flaxseed (Marambe, Shand, and Wanasaundara 2013) and potato (Gahlawat and Sehgal 1998). Protein digestibility often increases from seeds to flours and from flour to protein concentrates and isolates. Refining processes such as dehulling, defatting or degumming can increase protein accessibility by removing non-protein components such as mucilage, cell walls, oil fractions, or by purifying proteins. Significant improvements in protein digestibility have been obtained by removing anti-nutritional factors from potato flour (Gahlawat and Sehgal 1998), oil and fibers from flax (Marambe and Wanasaundara 2017). Heating processes such as cooking, baking, boiling or roasting greatly affect protein digestibility, which may be either improved or reduced depending on the conditions (Promeyrat et al. 2010; Wu et al. 1994). Indeed, heating can disrupt intra- and inter-molecular bonds thereby facilitating the unfolding of proteins, resulting in either increased solubility or aggregation, the latter often resulting in decreased solubility (Bax et al. 2012, Laleg et al. 2016, 2019). Moderate time and temperature treatments cause disaggregation and opening, both of which improve digestion, whereas when in excess, treatments produce indigestible aggregates (Promeyrat et al. 2010; Wu et al. 1994).

Combining the different components of protein nutritional value to calculate the food ration

Knowing the protein concentration (see 2.1), the EAA profile balance (see 2.2) and protein digestibility (see 2.3) makes it possible to calculate a daily recommended food intake (RFI) that meets both nitrogen and amino acid needs (Equation 5).

$$\text{RFI} = \frac{\text{RPI}}{\text{Protein concentration} \cdot \text{Protein digestibility} \cdot \text{LAAI}} \quad (5)$$

where RPI is the recommended protein intake (RPI)

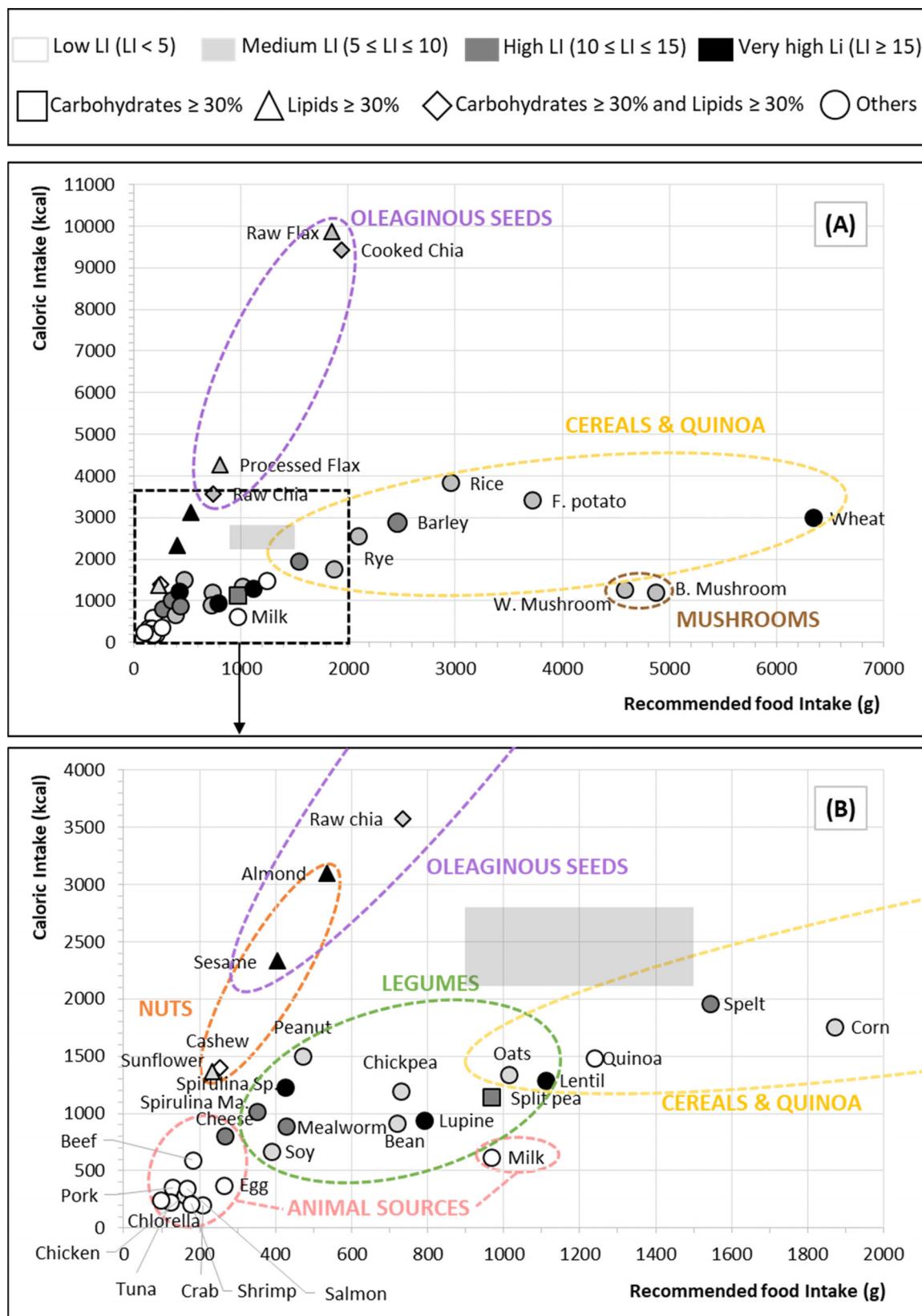


Figure 4. Caloric intake versus food intake for animal and alternative protein sources. **Figure 4-B** shows the enlarged area identified in the dotted rectangle of **Figure 4-A**. Loss index (LI) levels are indicated by the level of gray. Macronutrient dominances are indicated by the different shaped symbols (circles, squares, triangles). The large gray rectangle represents the daily food ration and caloric intake targeted.

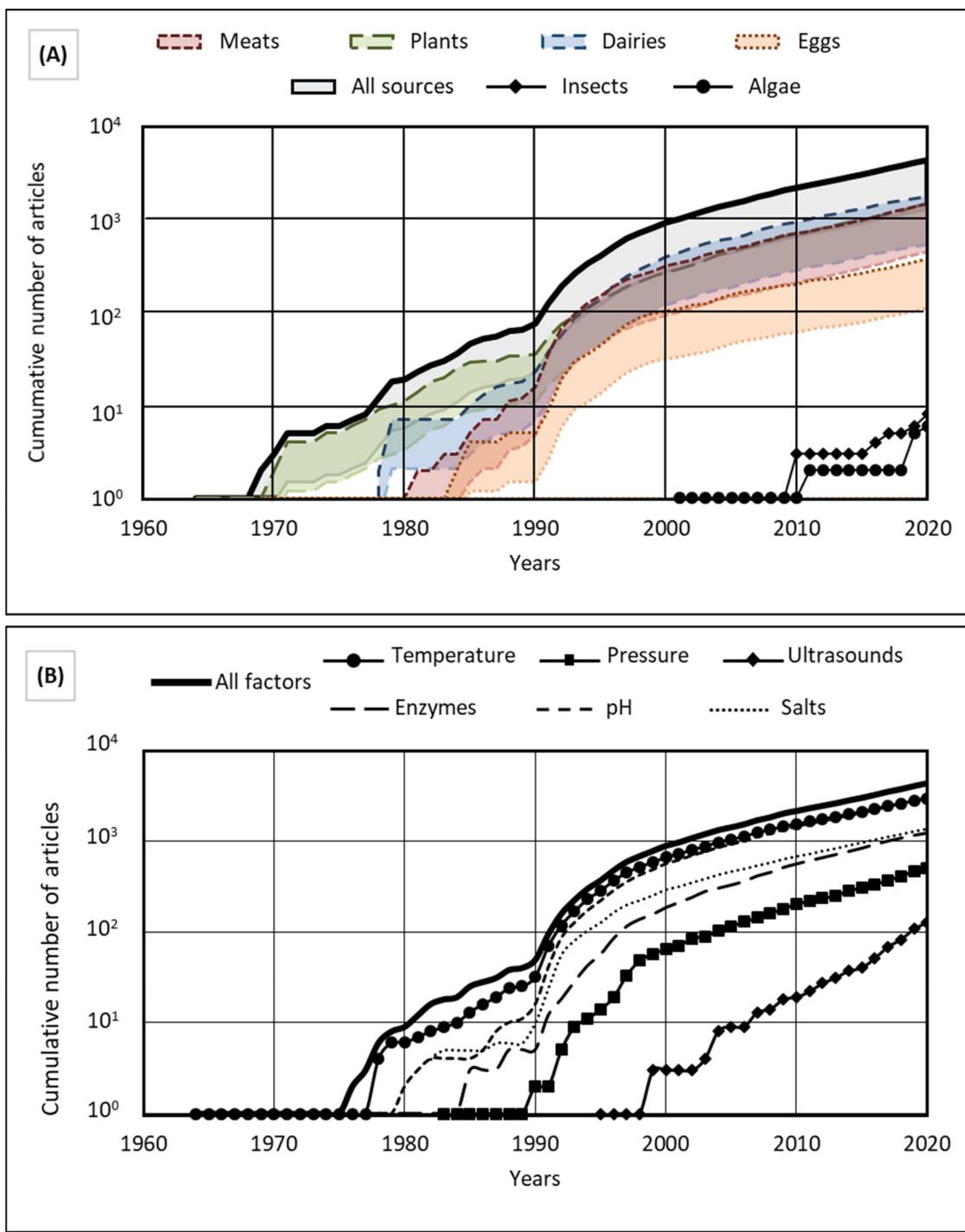


Figure 5. Annual cumulative number of articles referenced in the field "Food Science and Technology" of the Web of Science database covering the period 1945-2020 (A) according to the source of protein, including the proportion of potential off-topic papers for each (area is the same color as the corresponding curve) and (B) according to the process and/or the gelation conditions used.

corresponding to 0.66 g/kg/day of the reference protein, which, for a healthy adult weighing 70 kg, is 46 g/day (Afssa (Agence Française de Sécurité Sanitaire des Aliments) 2007).

We calculated the RFI of food materials in their final edible state, either raw (dairy, algae, etc.) or cooked (cereals, legumes, etc.) or both (nuts, oleaginous seeds, etc.) (Figure 3-A). In order to discuss the corresponding caloric intake, we also calculated the RFI of each food material (Figure 3-C) and showed their proportions of macronutrient (Figure 3-B). At this stage, we assumed that only one food source of

proteins would be consumed throughout the day to check if the corresponding quantity of food to be eaten was realistic (i.e., not too much, to avoid stomach distension). A reasonable daily reference food intake is in the range of 900-1,500 g of food per day, since people spontaneously consume 300 to 500 g of food per meal (Rolls, Morris, and Roe 2002). Daily reference caloric intakes for healthy young females and males with moderate activity are, respectively, 2,100 and 2,800 kcal/day (EFSA (European Food Safety Authority) 2016).

The daily RFI for foods containing animal proteins is well below the recommended range, with the exception of milk ($\approx 1,500$ g/day) and yoghurt ($\approx 1,000$ g/day) due to their higher water content. It is thus easy to meet the nutritional needs with an animal protein source completed with fibers (fruit, vegetables, and seeds) and vitamins (present and different in raw materials of vegetal origin). However, caloric intake is not sufficient in all cases. They contain very small amounts of carbohydrates or none at all (with the exception of milk and yoghurt, which contain lactose). Lipids, which are present in all animal source foods, are not sufficient to supply the energy required for proteolysis, amino acid use and protein synthesis (FAO (Food and Agriculture Organisation)/WHO (World Health Organisation)/UNU (United Nations University) 2007). Thus, a carbohydrate source has to be added in the diet, which is usually done all over the world using cereals, legumes and/or tubers.

The daily RFI values for foods containing proteins of vegetal origin are much higher because their protein contents are lower, they are less digestible and their amino acid contents are not all sufficient. Moreover, some of these vegetal protein sources are also rich in starch and in lipids, thus supplying complementary energy. The RFI of legumes, nuts and oleaginous seeds are below or in the range of reasonable food rations. However, the RFI of some other seeds, all cereal raw material and all tubers is sometimes much greater than 1,500 g, which is not acceptable in terms of daily ration. Yet, bread made of wheat flour, is in the reasonable ranges of both RFI and caloric intake due to the impact of processing on composition. Protein content plays an important role in the RFI. For example, cereals and tubers with a high RFI have lower protein contents. The proportion of lipids also plays an important role in caloric intake. Sunflower, rapeseed, almonds, and cashews indeed have a low RFI and high caloric intake, some well above 2,800 kcal. However, the EAA profile balance plays also a role. For example, the RFI of almonds is higher than the RFI of cashews even if almonds have higher protein contents (Figure 1, section 2.1). The reason is the difference in their EAA profile: the LI values of almond and cashew are 15.5 and 4.6, respectively (Table 3). The much higher LI for almond but also its higher proportion of lipids lead to a slightly too high caloric intake (3,000 kcal) while that of cashew is significantly lower (1,500 kcal).

Other alternative protein sources (insects, mushrooms, algae) have variable performances in terms of RFI. Despite their LI value of about 7-8 located in the "medium" category, mushrooms and fresh wakame have a very high RFI. This is due to their high-water content. Their use in a dried form may be a solution if the drying process does not reduce digestibility and does not degrade the balance and chemical integrity of EEA. For an insect like mealworm (even if its LI is higher than that of a cricket, Figure 2-H2, section 2.2), both the RFI and the resulting caloric intake are well below 900 g and 2,100 kcal, respectively. The protein content of such alternative sources is similar to that of animal sources of protein (Figure 1, section 2.1). However, like for animal protein sources, as insects mostly comprise protein and

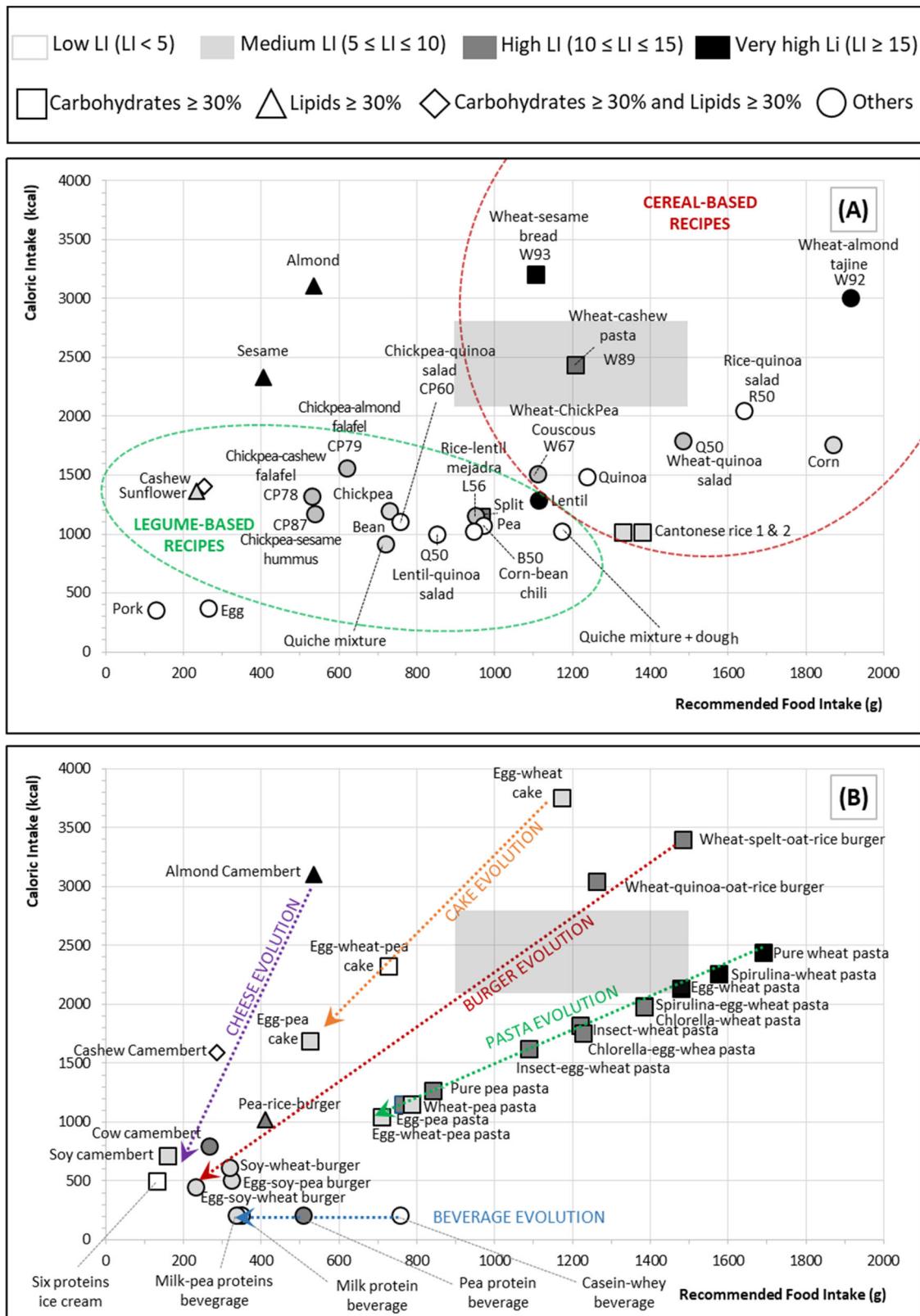
lipids, the addition of a carbohydrate source is necessary to reach the 2,100-2,800 kcal daily caloric intake. When dried algae are used, complementary sources containing high concentrations of these two macronutrients are necessary because of their low carbohydrate contents and very low lipid contents.

Therefore, when replacing animal proteins by proteins from other sources, one has to take both the quantity and quality of protein into account but also the effect of the process on the composition and digestibility of the protein. Even considering all these factors is still not sufficient. It is also necessary to include their impact on the daily ration, which needs to be ingested to cover human protein requirements. It should not be too high but just cover caloric needs, without exceeding them. Figure 4 gives a global view combining the characteristics and the nutritional constraints of all the protein sources with respect to their EAA balance and ration/caloric daily intake. At a glance, it is possible to identify the different sources and their distance from the targeted daily ration and calorie intake (gray rectangle). Oleaginous seeds are above the caloric intake, while cereals, mushrooms and tubers are above the RFI. Animal sources, legumes, and nuts are below the targeted food intake. Animal sources and legumes are also below the targeted caloric intake. For each protein source, the level of gray gives its LI level and the shape of the symbol the proportion of macronutrients. This type of representation makes it easy to work out if a daily diet contains animal protein or not, whether it meets all protein and caloric needs, and whether it optimizes protein use by the body (i.e., limiting LI).

Very few proteins from non-animal sources are, on their own, able to meet human EEA needs, but also needs for other micronutrients such as fatty acids, some vitamins (B12 for example) and iron. However, most proteins from non-animal sources provide a higher nutritional density (thanks to their fatty and/or carbohydrate composition) and some of them are good fiber sources. Moreover, as the EAA profiles and proportions in macronutrients differ, it is clear that mixing some of them could result in a more balanced EAA profile (optimal protein use by the body) and foods and/or a diet that provides the necessary daily food ration and reaches caloric intake targets. However, changing the protein content of a food might not only affect its nutritional interest but may also affect its sensory properties, especially texture. If nutritional and sensory aspects of protein-based products are often studied separately, both of them have to be considered when formulating food, since a change in either one of them can affect the other. Thus, before exploring different ways to design products with optimized EAA profiles, it is useful to review existing knowledge on the mechanisms and texturizing ability of proteins.

How to create food texture with proteins from different origins

Protein functional properties are used to structure food. Understanding and controlling these functional properties is essential when the aim is to replace animal proteins with



proteins from alternative sources, while preserving the sensory, nutritional and functional properties of the original product. The main functional properties of proteins can be sorted in two categories, both of which are able to provide texture and stability to complex food products. On one hand, thickening and gelling properties involve proteins, which are dispersed in a continuous phase and interact among themselves thereby contributing to the texture of the food. On the other hand, emulsifying and foaming properties involve amphiphilic proteins that migrate to the interfaces between two immiscible phases, one being dispersed within the other continuous one. Protein stabilizes this unbalanced system by helping to reduce the size of the dispersed droplets and by increasing the electrostatic repulsion between the dispersed elements.

All proteins are amphiphilic irrespective of their origin. Therefore, they have varied emulsifying or foaming ability, which can be either improved or limited by processing. However, not all proteins have thickening and/or gelling properties and processing influences these properties. As most animal proteins used in food products have gelling properties, when replacing them in food, knowledge is required of the gelling properties of proteins from alternative sources and the process used to bring them through a sol-gel transition. Given the number and the diversity of proteins, we searched the worldwide Web of Science database to see which proteins are able to gel and which processes are involved, depending on the protein sources.

Analysis of the scientific literature on food protein gelation

Our search was restricted to the “Food Science Technology” domain and covered the period 1945-2020. The search formula was extended to title, abstract and keywords and was implemented as described in the [Appendix](#).

[Figure 5-A](#) shows the cumulative number of scientific publications over time for each protein source. The residual noise, estimated on relevance analysis, is represented as colored areas, except for insects and algae since no off-topic publications were found after the excluded words were included. We found no articles dealing with gelation of mushrooms proteins. [Figure 5-A](#) shows that the gelling properties of food proteins have attracted increasing interest in the last 50 years, with more than 6,000 cumulated

publications in 2020. The papers published before the 1970s have generally rarely been digitized, which explains the absence of references older than 50 years in our search results. The shoulder, which is systematically present after 1990, corresponds to the beginning of the systematic digitalization of papers after this date. The same applies to [Figure 5-B](#). Nevertheless, the number of publications has greatly increased since the 1970s and, as the same treatment was applied for all protein sources, it is therefore possible to compare these cumulative curves. There are marked disparities between the fewer than 20 articles dealing with insect and algae protein sources, and the thousands of mentions of more traditional proteins of animal and vegetal origin. Indeed, plant, meat, milk, and egg proteins have been studied since 1970, whereas studies on insect and algae proteins only began in 2000, in the stressful context of increasing protein demand (Petrusán, Rawel, and Huschek [2016](#)). Already consumed for years in Asia (algae in miso or dashi) and Africa (roasted weevils), these “innovative” protein sources only recently appeared in Western markets and have gradually increased since. While the first products were quite simple and considered as side dishes (algae salad) or appetizers (grilled insects), a growing supply of more formulated and more familiar products is now appearing on the market, such as burgers made of microscopic mushroom (Quorn), insects (Essento, Entomi, Bug Foundation, etc.) or legumes (Herta, Findus, Fleury-Michon). In parallel with the boom in innovative protein products, proteins of vegetal origin remain of interest with a growing range of plant substitutes for burgers (Impossible Burger, Beyond Meat, etc.) and plant substitutes for dairy products. Given their presence on the market, there is an increasing need to improve our knowledge of their functional properties, including gelation.

[Figure 5-B](#) shows the cumulative number of publications dealing with protein gelation. Like [Figure 5-A](#), the rare digitalization before the 1970s explains the absence of references before 1976. However, from this date, and even after 1990 when digitalization became systematic, it is obvious that protein gelation was attracting growing scientific interest, with more than 5,000 papers published between 1976 and 2020. The four main conditions controlling gelation are temperature, pH, ionic concentration and enzymes, whereas publications on pressure and ultrasound are more recent and started in the 1980-1990s. Indeed, pressure and ultrasound are more difficult to implement since the necessary

Figure 6. Caloric intake versus daily food ration intake for mixtures of proteins from (A) traditional dishes and (B) industrial or research products. Dominant macronutrients are indicated by the different shaped symbols. Loss Index (LI) levels are indicated by the level of gray. The gray rectangle represents the daily food ration and caloric intake targets. Arrow heads indicate that the RFI and CI of the products come closer to those of animal sources. In [Figure 6-A](#), capital letters refer to the two protein sources of the mixture and number refers to the proportion of this protein source in the formula. A: Almond. B: Bean. C: Cashew or Corn or Chickpea. L: Lentil. Q: Quinoa. R: Rice. S: Sesame or Sunflower. W: Wheat. W93 means that the wheat-sesame bread contains 93% of wheat. In [Figure 6-B](#), recipes are based on information available on scientific publications or commercial products. Cakes recipes are based on (Monnet, Laleg et al. [2019](#), Monnet, Michon, et al. [2019](#)). Pasta recipes are based on pea-wheat pasta from (Petitot et al. [2010](#)), molitor-wheat pasta from Jimini's brand, spirulina-wheat pasta “Rubans Spiruline” from Lazaretti brand and transposition to chlorella. Burger recipes are based on Wheat-Spelt-Rice-Oat burger “Les Galettes, boulgour et épeautre” from Céréal brand, Wheat-Quinoa-Rice-Oat burger “Les Galettes, Boulgour et Quinoa” from Céréal brand, Wheat-Soy burger “Grill végétal, Steak soja et pois” from Céréal brand, Egg-Pea-Soy burger “Côté végétal, Steak soja et pois” from Fleury Michon brand, Egg-Soy-Wheat burger “Hachés façon burger” from Bjorg brand, Egg-Wheat-Soy burger “Le bon végétal, Steak soja et blé” from Herta brand and Pea-Rice burger “The Beyond Burger” from beyond Meat brand. Beverage recipes are based on pea-milk protein beverages from (Ben Harb et al. [2018](#)) and casein-whey beverage “Shape Shake Cookies & Cream” from Food Spring brand. Six proteins ice cream recipe is based on “So Shape ice cream Mint and Chocolate” from So Shape brand. Camembert recipes are based on personal simulations replacing 100% cow milk by 100% vegetal milk. When the exact information where not indicated on commercial products, rational assumptions were made to match the component order and the nutritional content.

equipment is often expensive, the scale is limited, and is not suitable for continuous use.

Depending on the source of the protein, the gelation functional property may be expressed or not, with different underlying mechanisms. These mechanisms are interesting to understand when considering replacing animal proteins in food.

Understanding gelation processes in terms of the origin, nature, and structure of the protein

Proteins from animal sources have been well studied, extracted and isolated, precisely identified with a name given to isolates and their gelling conditions are widely characterized. For example, the gelling proteins of milk are identified as casein micelles made of several kinds of caseins, and globular proteins like α -lactalbumin and β -lactoglobulin. Gelation of caseins occurs at pH 4.3. Gelation of lactoglobulins occurs during heating above 80–90 °C (Dagleish 1990).

Gelation conditions of animal proteins are well documented and are mostly related to conformation changes. Most animal proteins are globular and are sensitive to temperature or pressure denaturation. These physical conditions cause the disruption of intramolecular bonds and the exposure of internal reactive amino acids, allowing the reformation of intermolecular bonds and the aggregation of proteins to form a network. Globulins gel in neutral pH but variations in pH and ionic strength are often used to disrupt the internal electrostatic bonds or to induce local electrostatic repulsion within the proteins. This enhances the unfolding of the proteins but cannot be the only driving force behind protein gelation, since another factor is often needed to complete the unfolding and trigger the aggregation of proteins.

Some other animal proteins have a non-globular structure and require different conditions from globular ones to make gels. Collagen and muscle proteins are extended-chain proteins insoluble and amorphous in their fibrous state. Their highly organized structure is well described (Djabourov et al. 1985; Park 2005; Lanier, Carvajal, and Yongsawatdigul 2005). Collagen and muscle proteins solubilize when hydrolyzed in strong acidic or alkaline conditions, disrupting the electrostatic interactions which maintains the bundles of fibrils and fibers (Park 2005). Gelatin is made of extended chains. Mammal gelatin solubilizes when heated to more than 40 °C and gels when cooled below 30 °C (Haug and Draget 2011). Gelatin undergoes a disorder-order transition from coil to helix conformations and associates in junction zones stabilized with hydrogen bonds to build triple helices (Djabourov et al. 1985; Michon, Cuvelier, and Launay 1993). This leads to the formation of thermal reversible gels stabilized by hydrogen bonds. Gelatin appears to be limited to animal sources so such processes are not necessarily transposable to proteins from other sources. Muscle proteins undergo sol to gel transition when unfolded with thermal or pressure treatments, driven by hydrophobic interactions reinforced with hydrogen, calcium and disulfide bonds (Park 2005). Gelation may also be enabled at low temperature and

pressure with endogenous or exogenous transglutaminases which form bridges between glutamine and lysine amino acids (Tahergorabi 2017). Muscle structures have been found in insect flesh, so such processes may be transposable to insect meat although none of the nine articles we identified in our review reported such a mechanism.

Milk caseins are associated in micellar structures, which, in turn, are associated in micellar colloids, masking the hydrophobic zones at the center while exposing the hydrophilic zones in contact with water. Still the subject of debate, the structure of micelles is documented in detail in (Dagleish and Corredig 2012). It is hypothesized to be made of a core of α_1 , α_2 , β caseins stabilized with intermolecular calcium phosphate bonds, inside a hairy highly negatively charged layer of κ -casein, which induces steric and electrostatic repulsion. Milk casein gelation occurs when the repulsion forces at the surface of the micelles are neutralized, destabilizing the colloidal suspension and allowing the proximity of micelles (Dagleish and Corredig 2012). The first casein gelation process involves acidification with lactic fermentation or with the addition of organic acids or lactones. Acidification helps screen the negative charges on the surface of the micelles, thereby neutralizing the electrostatic repulsion. Acidic gelation occurs around pH 4.3 and affects both the external and internal structures of micelles, removing the negative charges from the internal caseins and hence releasing calcium ions into the solvent. This process leads to the formation of soft gels and is mostly used to produce yogurt or cream cheeses. The other casein gelation process involves the addition of two hydrolytic enzymes extracted from veal rennet, chymosin and pepsin, which specifically hydrolyze the surface hairy layer of κ -casein and thus reduce the steric hindrance. Enzymatic gelation does not affect the internal structure of micelles, calcium ions remain captured and even strengthens the gel through the creation of divalent bridges. This process leads to the formation of harder gels and is mostly used to produce cheese and clotted products. Casein gelation can also occur at pH 5.8 to 6.7 when heated with addition of divalent cations which form bridges between the surface negative κ -casein and thus allow the creation of a network (Balakrishnan et al. 2018).

Gelation conditions of vegetal proteins are of increasing interest, but to date few species have been studied in detail. Most vegetal proteins are globular and form gels in the same conditions as animal globulins. Gelation of vegetal globulins takes place under an increase in thermal or pressure in acidic conditions and allows the creation of cheese analogues like tofu (Arntfield and Maskus 2011; Zhang, Li, and Mittal 2009). The gelation of legume globulins is enhanced with divalent cations, which create salt bridges between lysine and arginine amino acids (EeMan, DeMan, and Gupta 1986) and with transglutaminase, which creates covalent bridges between glutamine and lysine amino acids (Arntfield and Maskus 2011). These conditions may be suitable for other lysine-rich vegetal proteins such as cashew, flax and chia. However, they appear to be less suitable for cereals, roots and other nuts and seeds that contain less lysine (Figure 2, section 2.2). Gelation induced by

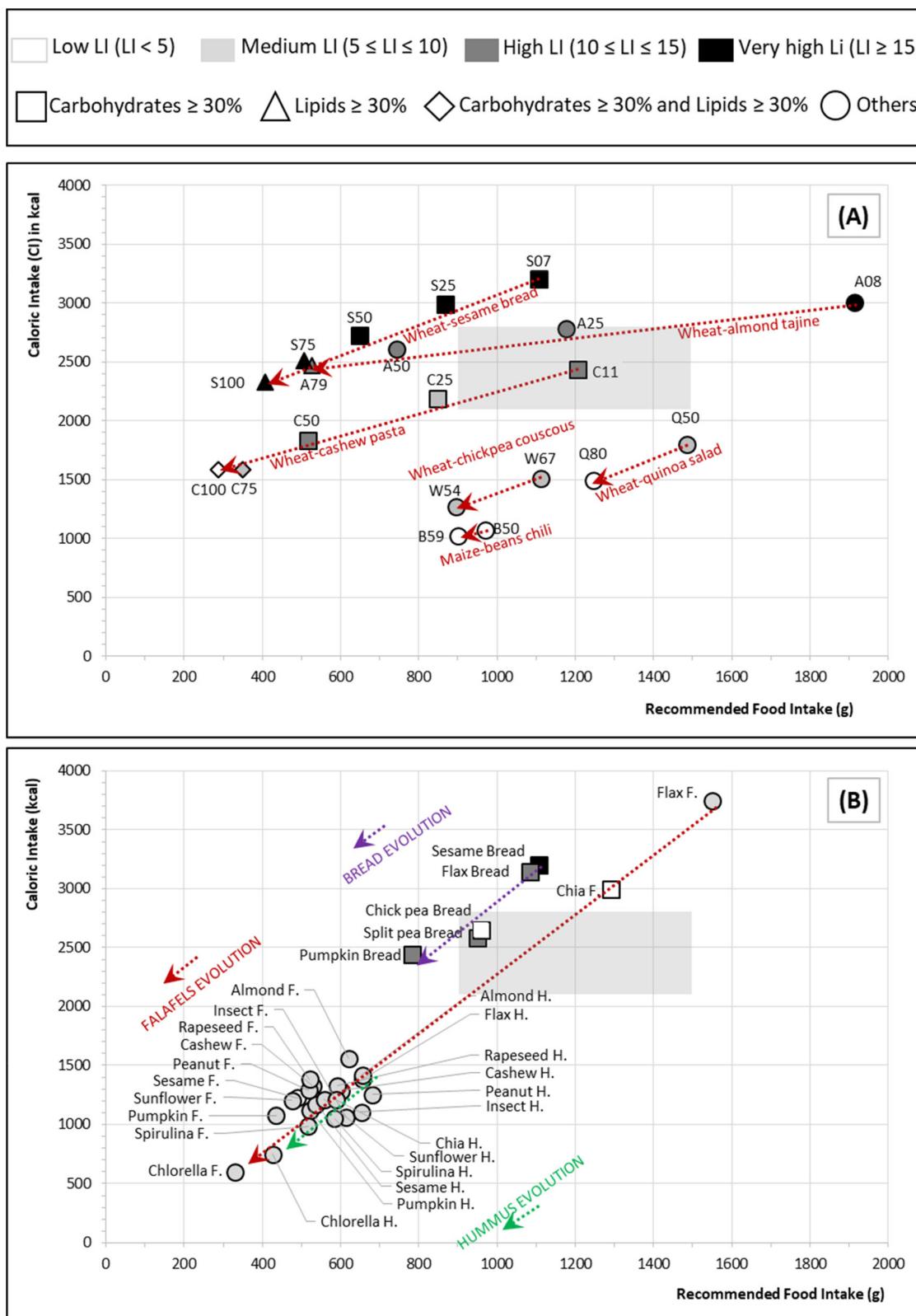


Figure 7. Optimization of the proportions of protein sources in traditional dishes and in industrial or research formulas, based on a minimum Loss Index (LI) and a realistic Recommended Food Intake (RFI) ≤ 1000 g. Loss index (LI) levels are indicated by level of gray. Dominant macronutrients are indicated by the different shaped symbols. The gray rectangle represents the daily food ration and caloric intake targets. Arrow heads indicate that the RFI and CI of the products come closer to those of animal sources. In Figure 7-A, capital letters refer to the two protein sources of the mixture and number refers to the proportion of this protein source in the mixture. A: Almond. B: Bean. C: Cashew or Corn or Chickpea. Q: Quinoa. S: Sesame. W: Wheat. S25 means that the wheat-sesame bread contains 25% of sesame. In Figure 7-B, capital letters indicate recipes. F: Falafel. H: Hummus.

temperature or by an increase in pressure has also been reported in cereals (Domenek et al. 2002; Arntfield 2011), nuts (Olalekan et al. 2009) and roots (Alting et al. 2011).

Wheat glutenin and gliadin proteins also display specific behavior when kneaded at room temperature (Van Vliet, Martin, and Bos 2002). The release and activation of

Table 4. Optimization of food and caloric intakes based on protein replacement and variations in the proportions of protein in high-protein ice cream and beverage. The underlined data are those from reference commercial mixtures, which are the starting point for optimization.

	LAAI	LI	RATION	PROT	GLU	LIP	CAL
High-protein ice-cream optimization							
6-protein ice cream	<u>1.33</u>	<u>3</u>	<u>132</u>	<u>47</u>	<u>39</u>	<u>12</u>	<u>497</u>
with milk isolates and spirulina, rice, oat, flax							
2-protein ice cream with soy and sesame proteins	1.11	4	114	43	33	10	444
High-protein beverage optimization							
2-protein beverage	<u>1.52</u>	<u>5</u>	<u>757</u>	<u>70</u>	<u>6</u>	<u>0</u>	<u>210</u>
with milk proteins							
2-protein beverage	0.99	6	639	84	6	0	210
with soy isolate and							
with sesame isolate							
2-protein beverage	1.00	6	630	84	6	0	207
with soy isolate and							
with sunflower isolate							

Table 5. Comparison of three daily diets containing different protein sources that meet the food and caloric intakes target. 500 g fruit and vegetables, which are not mentioned in the table, need to be added to fulfill human fiber and vitamin needs.

	Protein source	Food weight (g)	Proportion during the day	Calories kcal	Food Weight (g)	Calories (kcal)
European diet						
Breakfast	cow's milk	100	9%	68	250	512
	bread	150	14%	443		
Lunch	beef steak	100	9%	316	380	856
	pasta	180	17%	254		
	cow's milk camembert	100	9%	286		
Dinner	Salmon	100	9%	194	430	582
	rice	180	17%	238		
	cow's milk yogurt	150	14%	150		
Day	total	1060	100%	1950	1060	1950
Lacto-ovo-vegetarian diet						
Breakfast	cow's milk camembert	80	8%	229	230	413
	oats	150	14%	184		
Lunch	almonds	100	9%	596	450	904
	couscous	100	9%	136		
	cow's milk	250	24%	171		
Dinner	almonds	120	11%	185	380	525
	wheat-pea pasta	160	15%	240		
	cow's milk yogurt	100	9%	100		
Day	total	1060	100%	1841	1060	1841
Vegan diet						
Breakfast	soy camembert	100	11%	412	220	559
	oats	120	13%	147		
Lunch	pea-rice burger	120	13%	300	350	841
	couscous	150	16%	205		
	cashew cheese	80	8%	337		
Dinner	almonds	30	3%	179	380	646
	wheat-pea pasta	200	21%	300		
	soy yogurt	150	16%	167		
Day	total	950	100%	2046	950	2046

intrinsic oxidase and isomerase break the internal disulfide bonds thus allowing the reaction of hydrogen and hydrophobic junction zones stabilized with new disulfide bonds. This behavior is specific to glutenin and gliadin proteins and cannot be transposed to proteins from other origins.

The gelation of protein from sources such as insects, algae and mushrooms, is still very rare or undocumented compared to that of protein of animal and even of vegetal origin. Few of these alternative proteins have been isolated and characterized and most are still considered as a set of undifferentiated protein with no precise name. There is still no gelled food derived from these protein sources, even if lots of the new products that appear on the market incorporate it as a nutritive raw material in more traditional products, such as biscuits (for example granolas enriched in

insect protein) or sauces (for example mayonnaise enriched in chlorella). There are still only a few technological levers, such as thermal, physical, chemical or biochemical processes, known to successfully thicken or gel these proteins. Thermal processes are still the most commonly applied methods in a variety of chemical conditions.

When the texturing functions are not present for some proteins of non-animal origin, the easy way is often to add gelling or thickening additives. The use of complementary polysaccharides is common. Indeed, some of them like agar-agar, carrageenan, pectin, xanthan and guar are well known gelling or thickening agents. This formulation strategy is not very clean-label as it increases the list of ingredients. Moreover, the addition of polysaccharides to food products leads them to enter the category of ultra-processed products

of the NOVA classification proposed by Monteiro et al. (2018). These ultra-processed products are now widely criticized for their potentially negative effects on health. It is therefore imperative to avoid the use of these additives as much as possible and to focus on formulating products using only native raw materials. Keeping good sensory and rheological properties without incorporating additives is very complicated. Much research focus is put on a detailed understanding of the interactions between components in the matrix during the transformation process, in order to optimize the expression of the texturizing functionalities of each of the naturally present protein or polysaccharide components. As an example, starch which is naturally present in many vegetal species (cereals, legumes and tubers) may increase the viscosity of the product when heated to 80 °C in the presence of water due to gelatinization. Starch granules swell and act as real internal water pumps. In this way, they occupy most of the volume, while the concentration of the other components that surround them increases markedly. This phenomenon leads to - at least - a large increase in viscosity, even if no true sol to gel transition occurs.

Thus, given the importance of food protein functionalities, the replacement of animal protein sources when formulating foods has to be done regarding their impacts on food structure and texture, in order to preserve the technological (manufacturing and cooking abilities) and organoleptic properties of the product but also the list of ingredients which must be as short as possible.

Innovative applications of non-animal proteins

Assessment of traditional recipes and innovative products

Among protein sources, combinations of two or more proteins of vegetal origin are often recommended for foods or dishes in vegetarian or vegan diets, to ensure the nutritional needs in terms of essential amino acids are met. Interestingly, this is what our ancestors did all around the world, formulating traditional dishes like Mediterranean couscous (wheat with chickpeas), tajine (wheat with almonds) and hummus (chickpeas with sesame), American chili (rice, corn or wheat with beans), or Indian mejadra (rice with lentils) and so on. Recently, rather classical foods like cakes, pasta or cheese have been reformulated to include another protein or to replace part of the usual protein source with another to improve the EAA balance. To discuss their relevance, we studied some examples of traditional and industrial multi-protein sources recipes. They are reported in Figure 6 using the same global view that, like in Figure 4 (section 2.4), combines all the characteristics and the nutritional constraints of protein sources with respect to the EAA balance and the daily ration/daily caloric intake.

As can be seen in Figure 6-A, cereals and legumes are often mixed but also combined with nuts or other seeds in traditional recipes. The corresponding pure protein sources need between 2,000 to 6,600 g (cereal) up to 10,000 kcal (flax and chia) to cover human needs (Figure 4, section 2.4). The two-protein mixtures are all located in a narrower range of

food intake (below 2,000 g) and caloric intake (below 4,000 kcal). The addition of other vegetal proteins to cereal-based recipes reduces both the food ration and the caloric intakes. However, some of the corresponding traditional dishes still supply too high food ration and/or caloric intakes and a very high loss index (wheat-almond tajine and wheat-sesame bread). The daily diet cannot be composed of only the latter dishes. The addition of vegetal proteins from other sources to legume-based products means dishes can be produced with lower caloric intake than the target (chickpea-cashew or -almond falafel, chickpea-sesame hummus, rice-lentil mejadra, corn-bean chili, etc.). Moreover, the food intakes of these dishes vary from 500 to 1,000 g, which are all equal to or below the reasonable daily food intake. In all cases, with these legumes-based recipes, the required protein would be covered by eating only this dish with minimum or medium LI. As can be seen in Figure 6-B, protein combinations are also used in industrial and research formulas, which either aim to increase protein content or to replace one protein source with another. Some authors studied how to replace wheat semolina and wheat flour with legume flour in pasta and cake, respectively (Petitot et al. 2010; Monnet, Laleg et al. 2019; Monnet, Michon, et al. 2019). Moving from traditional formulas with only wheat and possibly egg to formulas in which all the wheat is replaced by legumes reduced both the food ration and caloric intakes, with, in between, recipes that go beyond the target. At the same time, LI decreases from the range 10-13 to about 6 for pasta and from 3 to 2 for cakes. The introduction of chlorella, spirulina or molitor protein in wheat, pea or wheat-pea pasta, improves all the nutritional indicators of the original mixture. Indeed, although the corresponding proteins have the same LAAI, these protein sources contain higher protein concentrations than wheat and help reduce the food ration and caloric intakes. From a functional point of view, the gelling ability of the cake batter and of pasta is provided both by gluten denaturation and by egg globulins that interact when heated. In both cases, these properties can be at least partly replaced by globular proteins from legumes, provided that the mixing, the drying temperature and cooking processes are adapted (Dewaest et al. 2017; Petitot et al. 2010). In any case, the presence of a large amount of starch in both products, whatever the cereal or legume sources, helps solidification after thermal treatment.

Alternative cheeses based on vegetal proteins reduce the food ration and caloric intakes and the LI when changing from almond to cashew proteins and from cashew to soy proteins. This change can be explained by both their different EAA profiles and protein content. Soy contains more protein than cashew (38% versus 19.5%) and has a better-balanced EAA profile than almond (LI of 9-10 and 15, respectively). Interestingly, the LI of soy camembert is slightly lower than that of camembert made from cow's milk. However, it should be noted that we only found amino acid data on raw sources of proteins of vegetal origin whereas amino acid data on fermented cheese were available and were used for cow's milk camembert. Studies on how fermentation processes can influence the nutritional

performance of proteins are still rare, especially considering vegetal proteins. However, a similar impact is expected for vegetal proteins as for animal ones, with modifications of digestibility and amino acid profile, but it is difficult to know if this will improve or degrade the nutritional profile of vegetal proteins (Çabuk et al. 2018; Berrazaga et al. 2019; Steinkraus 1994; Yousif and El Tinay 2000). However, whatever the improvement or degradation, the positioning in Figure 6 would not change completely. From a functional point of view, chemical-induced gelation takes place in vegetal globulins as well as in milk proteins, induced under acidification and reinforced in the presence of divalent cations building bridges. Therefore, the formulation of vegetal cheese could probably be transferred to other vegetal globulin sources such as cereals (oat milk, spelt milk, rice milk), legumes (pea milk or peanut milk) and nuts (chestnut milk or hazelnut milk).

Vegetarian burgers are also available in the market. Recipes containing mainly cereal components, even when several species are used, have rather too high caloric intake and medium or high LI. On the other hand, recipes based on legumes (sometimes containing cereals as a complementary component) represent a very low food ration and caloric intake similar to that of beef burgers with only a slightly higher LI. The addition of egg can reduce the food ration and caloric intakes a little more. Eggs can be used as single protein sources in a diet but on their own, do not meet the required food ration and caloric intake. Used with bread (as a complementary starch source), tomatoes, onions and pickles, they can meet the daily food and caloric intake. Another possible way would be to use different legume/cereal ratios, which would help reduce the LI. It should be noted that other ingredients are often used in vegetarian burgers, probably to help achieve solidification. Methylcellulose is used to improve viscosity when mixing ingredients and gelling at high temperature. Starch supplied by the legume and cereal flours, is also present in these burgers. During pasting, the starch absorbs water, thereby increasing the concentration of protein and other carbohydrates around the starch granules helping to improve the texture of the burgers. By adjusting the proportion of each ingredient, it is probably possible to avoid using a thickening or gelling agent like methylcellulose.

Considering protein-enriched beverages or ice creams, the examples we reviewed showed good performance in terms of food ration and caloric intakes and LI. The 6-protein ice cream contains six different proteins originating from animal, plant and microalgae (which may also contribute color): milk powder, milk protein, rice flour, oat flour, flax, spirulina, oil and water. This product also contains quantities of texturizing additives with either thickening (maltodextrins and other fibers) and/or gelling properties (like xanthan and guar together according to Cuvelier and Launay (1985)). The 6-protein ice cream represents a much lower food ration intake and a lower LI than the 2-milk protein beverage, suggesting that combining such proteins leads to a better-balanced essential amino acid profile. However, the ice cream still contains animal proteins and globally a

large number of ingredients that are less and less acceptable to today's consumers. Another example is a milk-pea protein beverage in which half the milk protein was replaced by pea proteins (Ben Harb et al. 2018). Its food ration intake is lower than the casein-whey beverage and the pea protein beverage ones. Its LI, being in the medium range, is better than pea protein alone.

Rational optimization of recipes containing no animal protein

Considering the examples above that combine two protein sources, we estimated optimal proportions of ingredients to allow a minimum LI and a RFI in the range 900-1,500 g. Optimization was performed using the protein concentrations and the EAA profiles of either raw or cooked materials, but always in the edible state, since food processing can influence the nutritional value of food proteins. The resulting products are shown in Figure 7 along with their food ration and caloric intakes indicating their LI levels and macronutrient proportions.

It is possible to reduce the food intake of bread and tajine by adding sesame grains and almonds, respectively (Figure 7-A). However, the caloric intake of both these products, consumed at the ration containing the daily protein human need provides a rather high level of calories and the LI decreases only slightly for tajine. Moreover, adding sesame or almond means the product will contain a high concentration of lipids. Tajine containing 25 wt % of almonds seems to be a good option as long as it is not the only food consumed in the day. For example, it could be completed by a 200 g. pea-rice burger. Only 600 g of tajine with 25% of almond would be necessary. The total daily food portion would be 800 g and would provide 1,900 kcal.

Both food ration and caloric intakes in wheat-chickpea couscous, wheat-quinoa salad and corn-bean chili can be reduced simply by altering the proportions of the two components (Figure 7-A). Moreover, their LI all decrease and, in some cases, can reach a low LI level. The addition of 100 g of raw flax to 850 g of wheat chickpea couscous or 200 g of cooked chia to 810 g of corn-bean chili, makes it possible to reach the daily food and caloric intake target.

Increasing the proportion of cashew in wheat-cashew pasta reduces the food and caloric intakes and the LI value (Figure 7-A). However, a high proportion of cashew in the pasta makes it difficult to produce the pasta. Adding cashew as a side dish in the sauce would be easier. Peanuts, raw or cooked flax, raw or cooked chia, or almonds are other interesting options. With the same logic, other optimisations are proposed in Figure 7-B for bread, falafels and hummus using different protein sources including algae, mushrooms and insects.

Finally, optimization of both the food and caloric intakes and LI was performed for the 6-protein ice cream and the 2-protein beverage containing milk proteins. The resulting proposals are listed in Table 4. Rather low LI was obtained for both the ice cream and the beverage. Interestingly, with only two protein sources (soy and sesame), an ice cream

with very similar LAAI, LI, ration and caloric intake and macronutrient proportion was obtained. Two recipes for a vegan 2-protein beverage, with LAAI, LI, ration and caloric intake and macronutrient proportions very similar to the milk reference are included. These two types of products could be used for high protein diets adapted for athletes or for older people.

Conclusion

Animal proteins can be replaced by proteins from alternative sources, but most alternative sources do not meet human EAA needs in terms of their EAA profiles and/or their digestibility. As the EAA profiles differ, understanding them is useful to choose complementary profiles and formulate new or optimized products. In the future, when protein sources become limited with respect to the world population, limiting the loss of proteins due to EAA unbalance in the diet will be a major advantage. The ability of alternative proteins to contribute to the texture of the food products should also be included in the formulation process either by adapting the transformation process in order to optimize the functionalisation of proteins and polysaccharides naturally present, or by selecting additional non-animal protein sources with complementary EEA profile and texturizing abilities. To replace animal proteins, mixtures of proteins from alternative sources are preferably needed. The choice and proportion of food sources should be based on their protein concentration, EAA balance and protein digestibility, and should integrate the process which can have a major impact. Thus, we defined a new index called “Recommended Food Intake” which indicates the necessary food amount to eat to meet the protein needs regarding all these aspects, and another index called “Caloric Intakes” which estimates the corresponding caloric intake. A global representation including food and caloric intakes but also the loss index and the proportions of macronutrients will help predict the effects of reformulation, but will also help choose different protein-containing foods in a diet that meets the human need for protein and caloric intake. This nutritional tool can then be useful to assess or define realistic protein diets adapted to the needs, depending on the characteristics of the target population (without forgetting the other important nutrients for certain populations). In this review, most formulations based on alternative proteins aim to come closer to animal proteins, with a view to increasing the LAAI, reducing the RFI and reducing the CI. However, the same work can be done with another nutritional purpose, like increasing both protein and caloric intakes in populations that generally reduce their food intake, such as elders, or increasing both protein and food intakes while reducing caloric intake for people who are on diet. Table 5 gives examples of composition for three different diets (European, lacto-ovo-vegetarian and vegan). All three provide the appropriate quantity of protein, optimize EAA loss (medium global LI) and equivalent ration and caloric intakes.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix

Research methods found in our review of scientific literature analysis on protein from all sources, gelation properties and conditions

The general query pattern was defined as follows with two keyword categories corresponding to (1) Product or Protein (2) Gelation process and (3) Exclusion words. Boolean connectors combined the keywords using, when necessary, excluded words (Equation A1).

$$\text{TS} = (\text{Protein\$}) \text{ AND } (\text{gelation}) \text{ AND } (1) \text{ AND } (2) \text{ NOT } (3) \quad (A1)$$

Keywords were added either using a \$symbol to search for both singular and plural or a * symbol to search for all words derived from the root to avoid writing long formulas. Thus, the search

protein\$ searched for both *protein* and *proteins* and the search *proteoly** searched for both *proteolysis* and *proteolytic*. Particular attention was paid to monosyllabic keywords declined in off-topic keywords with the same root and were replaced with both singular and plural form instead of * to avoid off-topic articles. Thus, *pea or peas* was better than *pea** (*peasant, peanut, etc.*) or *pea\$*(*peak, pear, etc.*). Keywords were selected to include a maximum number of on-topic publications, including raw materials (such as *milk*), final products (such as *tofu*), species (such as *wheat*) and proteins (such as *gluten*). Special care was taken to avoid off-topic papers particularly with keywords related to gelation. Indeed, the keyword *gel* would include all the off-topic publications dealing with methods like gel filtration, gel electrophoresis, gel chromatography. Therefore, the words *gel* and *gels* were not used nor all the associated forms such as *gel formation, gel construction, gel structure, gel texture*. Moreover, the data base did not differentiate between the noun *gel*, the verb *gel* and its conjugated forms *gels, gelling, gelled* which produced the same results as *gel*. Thus, these forms were not used nor all the associated forms such as gelling process, gelling ability, gelled products, gelled proteins. Additional searches confirmed that these forms were accompanied by the word *gelation* in most of the publications concerned. They were also selected only using the keyword *gelation*. Keywords related to off-topic fields were excluded thanks to the *not* operator to avoid articles dealing with agronomy, microbiology, toxicology, chemistry, health, nutrition and others. Additional searches showed that exclusion words could exclude both irrelevant and relevant article and had to be used with caution to ensure that only irrelevant results were excluded. Therefore, exclusion keywords were used only when the number of publications allowed us to check each result with and without each exclusion word to ensure all relevant article would be included. Search results were sorted by year and a total of 100 results were sampled including 50 older results

and 50 newer results. Each publication among this sample of 100 references was studied to determine whether it was on-topic or off-topic with particular attention paid to the title and keywords, to the abstract when available, and the whole article when accessible. This allowed us to calculate the main residual proportion of off-topic publications that could not be avoided without losing very interesting papers. It was in the range 17 to 25% depending on the protein sources.

Table A.1 lists the keywords used for each origin of gelled food and gelling protein from a wide range of sources. Additional keywords were also selected when representative products (such as "tofu") and proteins (such as "vicilin") were known and more likely to be used instead of a general word (like "vegetal" or "legume"). No animal species keywords were accepted since they could be related to meat, dairy and eggs. No dairy product keywords were accepted since they could be related to both animals and plants. Only the keyword *milk* was kept since it would have excluded too many relevant papers that mention the word *milk* but not the related proteins or products.

Table A.1 also lists the keywords selected for each condition commonly used to produce gels in food. Our first searches revealed that the conditions play different roles in the gelation process and allow either protein solubilization, protein denaturation or protein interaction and network implementation. Some of them, such as temperature, were sufficient to enable gelation and could thus be used as the only condition. Others, such as ionic strength, do not allow gelation unless they are combined with at least one other condition. However, these environment conditions are useful to solubilize proteins and/or to denature proteins and to control the gelation point when the first endless aggregate appears. Regardless of the gelation conditions, gel formation was also subject to minimum water concentration and/or minimum protein concentration. Few articles were found that did not mention concentration, so this word was not used for searches.

Table A.1. Keywords related to food products, food proteins and processes involved in gelation.

Class	Class keywords	Species keywords	Protein keywords	Product keywords
VEGETAL PROTEINS INVOLVED IN GELATION				
Legumes	legume* OR soy* OR pea OR peas OR bean\$ OR chickpea\$ OR peanut\$ OR lentil\$ OR lupin\$ OR glycinin\$ OR conglycinin\$ OR vicilin\$ OR convicilin\$ OR tofu OR tempeh			
True cereals	cereal\$ OR wheat\$ OR corn\$ OR maize\$ OR rice\$ OR oat\$ OR buckwheat\$ OR spelt\$ OR barley\$ OR rye\$ OR millet\$ OR fonio\$ OR sorghum\$ OR fonio OR sorghum OR gluten OR glutenin\$ OR glutelin\$ OR prolamin\$ OR gliadin\$ OR avein\$ OR secalin\$ OR zein\$ OR kafirin\$ OR hordein\$ OR seitan			
Pseudo cereals	buckwheat\$ OR quinoa\$ OR amaranth*			
Other seeds	flax\$ OR flaxseed\$ OR linseed\$ OR chia\$ OR hemp\$ OR (squash AND seed\$) OR (gourd AND seed\$) OR (pumpkin AND seed\$)			
Nuts	-	Nut\$ OR hazelnut\$ OR walnut\$ OR chestnut\$ OR pinenut\$ OR almond\$ OR cashew\$ OR pistachio\$		
Tubers	-	root\$ OR tuber\$ OR potato OR potatoes OR manioc\$ OR yam\$ OR cocoyam\$ OR taro\$ OR patatin		
ANIMAL PROTEINS INVOLVED IN GELATION				
Flesh	meat\$ OR fish\$ OR fishery\$ OR muscle\$ OR myofibril\$ OR myofilament\$ OR myosin\$ OR actin\$ OR troponin\$ OR tropomyosin\$ OR gelatin\$ OR collagen\$ OR tropocollagen OR surimi			
Milk products	Dairy* OR milk* OR Whey\$ OR casein\$ OR lacto* OR lacta* OR Cheese\$ or yogurt*			
Egg products	egg\$ OR ovomucin\$ OR ovomucoid\$ OR ovalbumin\$			
INNOVATIVE FOOD PROTEINS INVOLVED IN GELATION				
Insects	insect\$ OR cricket\$ OR grasshopper\$ OR mealworm\$ OR worms\$ OR caterpillars\$ OR weevil\$ OR (tenebrio AND molitor) OR (alphitobius AND diaperinus) OR (lucosta AND migratoria) OR (acheta AND domestica) OR (acheta AND domesticus)			
Algae	-	microalgae\$ OR algae\$ OR seaweed\$ OR spirulina\$ OR chlorella\$ OR wakame\$ OR nori\$		
Mushrooms	fung\$ OR mushroom\$ OR myc*			
PROCESSES INVOLVED IN PROTEIN GELATION				
Temperature	temperature* OR thermal* OR heat* OR cool*			
Pressure	pressure*			
Sonication	sonic* OR ultrasound*			
Enzymatic activity	rennet# OR pepsin# OR chymozin# OR trypsin# OR chymotrypsin# OR proteolysis OR proteolytic OR proteolytically OR hydrolysis OR hydrolytic OR hydrolytically OR peptidase# OR proteinase# OR gelatinase# OR glutaminase# OR transglutaminase#			
Hydrogen activity	pH OR acid* OR base OR bases OR lactone* OR gluconolactone*			
Ionic activity	salt* OR mineral* OR ion* OR cation* OR anion* OR electrolyt*			