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Effect of Temperature on the Hydration Kinetics of Chickpea (*Cicer arietinum* L.) and Yellow Soybean (*Glycine max*)

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Within the final goal of reducing the anti-nutritional factors in chickpea (*Cicer arietinum* L.) and yellow soybean (*Glycine max*) by means of their malting process, the main aim of this work was to study the kinetics of water hydration of such seeds at different temperatures in the range of 12-36 °C for as long as 24 h. The kinetics of such a process was reconstructed using the well-known Peleg model. The Peleg rate constant (k_1) decreased with increasing temperature and was described by an Arrhenius type relationship. The estimated activation energy was equal to 36±6 or 60±3 kJ mol^{-1} for chickpea or yellow soybean, respectively. The Peleg capacity parameter (k_2) was approximately constant and led to an equilibrium moisture ratio of 1.4 or 1.8 g of water per g dry matter for the above seeds. A 5-h soaking at T≥24 °C rehydrated both seeds up to a moisture content of ~50% (w/w), this being preliminarily assessed as sufficient for activating the metabolic processes of germination.

1. Introduction

Legumes are an excellent source of protein, dietary fibers, and micronutrients, and are often identified as useful replacers for foods of animal origin in the diet thanks to their nutritional profile, and low cultivation costs and environmental impact (Nemecek et al., 2008; Coles et al., 2016). Among them, soybean (*Glycine max*) is the legume most largely produced worldwide with about 336.6 million metric tons (Mg) in 2019-2020, 36.5, 28.7, and 15.7 % of which being cultivated in Brazil, the United States, and Argentina, respectively (SOPA, 2020). The United States and western countries mainly utilize soybean indirectly in the food supply as livestock feed (i.e., soybean meal) or as soy oil and food ingredients, such as textured vegetable proteins and protein isolates. For this reason, soybean is not classified as pulse but as oilseed (Rawal and Navarro, 2019). On the contrary, the Eastern Asia population uses whole soybeans and processed (fermented and non-fermented) soy foods daily (Shea et al., 2020). Among the pulses primarily grown for human consumption (whole seed as such or dehulled, and flour), common beans and chickpeas (*Cicer arietinum* L.) are the legumes mostly produced in the world, their production in 2014 totaling to 24 and 13 million Mg, respectively (Rawal and, Navarro, 2019). About 74 % of the overall chickpea production is concentrated in South Asia, India being by far the largest producer of chickpea (67 %), followed by Australia (5.9 %), and Pakistan (4.6 %), as reported by Rawal and, Navarro (2019).

Despite the relatively high protein content of pulses, their protein quality is low owing to a small percentage of sulfur-containing amino acids. Some anti-nutritional factors, such as phytic acid, lectins, and tannins, exert a negative impact on the nutritional quality of the protein, interfere with the absorption of nutrients, and reduce protein digestibility and mineral bioavailability (Gilani et al., 2005). Oligosaccharides, that is low-molecular weight non-reducing sugars like stachyose and raffinose, induce flatulence, while trypsin inhibitors in soybean reduce protein digestibility (Mohan et al., 2016). Such compounds are partially degraded through traditional preparation methods, such as wetting followed by long cooking (Han et al. 2006). Alternatively, a malting process can lower their content and improve the sensory characteristics of legumes (Nkhata et al., 2018). The process of malting comprises three different unit operations: steeping, germination and drying. During steeping, seeds are immersed in water until imbibed with sufficient water to start their sprouting process. The most important physiological processes associated with the germination phase are the synthesis of amylases,

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proteases, and other endogenous hydrolytic enzymes, these being controlled by the moisture content of seeds, temperature, germination time, and oxygen availability. Drying is the final stage of the malting process, being required for stopping further growth of the germs, reducing water activity, and so producing a shelf-stable product with active enzymes. Dried seeds can be milled into malted flour ready for use in the preparations of different food products. Since barley kernel steeping greatly affects the final malt quality and relative water uptake rate is used to optimize the malting process (Gruwel et al., 2009), the main aim of this work was to study the kinetics of water hydration of chickpea and yellow soy seeds in the range of 12-36 °C for as long as 24 h, as a preliminary step to their germination, final drying and milling to obtain low-phytate and α-galactosides containing malted flours.

2. Materials and methods

Chickpea (*Cicer arietinum* L.) and yellow soybean (*Glycine max*) samples were purchased from a local supermarket in Viterbo (Italy). Both pulses were organically cultivated. The former was produced by the Azienda Agricola Monte Castello (Serravalle di Chienti, MC, Italy), while the latter was imported from the USA and packed by Borghini Srl (Arezzo, Italy). According to their commercial labels, dried chickpea composition was as follows: moisture (0.11 g g^{-1}), raw protein as Nx6.25 (0.202 g g^{-1}), fat (0.063 g g^{-1}), carbohydrates (0.469 g g^{-1}), and total fibers (0.136 g g^{-1}); while yellow soybean samples had the following composition: moisture (0.11 g g^{-1}), raw protein (0.369 g g^{-1}), fat (0.191 g g^{-1}), carbohydrates (0.232 g g^{-1}), and total fibers (0.119 g g^{-1}). The hydration kinetics of both legume kernels was studied in a bench-top plant, appropriately designed (Figure 1a). It consisted of an insulated chamber provided with 6 stainless-steel perforated baskets (Figure 1b), which altogether contained up to 1 kg of seeds during the wetting process. The relative humidity (RH) and temperature (T) of the air in the chamber were measured using a sensor type CJMCU-1080 HDC1080 (Texas Instruments, Dallas, Texas, USA) having an accuracy of ±2 % RH and ±0.2 °C, respectively. The temperature of the seeds was continuously monitored using a temperature probe type DS18B20 (Maxim Integrated, San Jose, CA, USA) having an accuracy of ±0.5 °C for a temperature range of -10 °C to +85 °C. The air ventilation inside the chamber was assured by two 120-mm fans type ARCTIC F12 (Artic Gmbh, Braunschweig, Germany), powered by an electric motor (DC 12 V), these assuring a nominal air flow rate of 90.1 m^3 h^{-1} at 1,350 rev min^{-1}. An aluminum (27.4x11.8x3.1 cm) radiator was used to control the air temperature by throttling or opening a valve feeding water cooled at the steeping temperature via an external chiller. The same cold water was also dispersed inside the chamber throughout the seed wetting process. Compressed air at 1.5 bar was continuously blown from the bottom of the chamber to aerate the seeds. A drainage system consisting of a solenoid valve and a filter was used to empty the chamber at the end of the process.

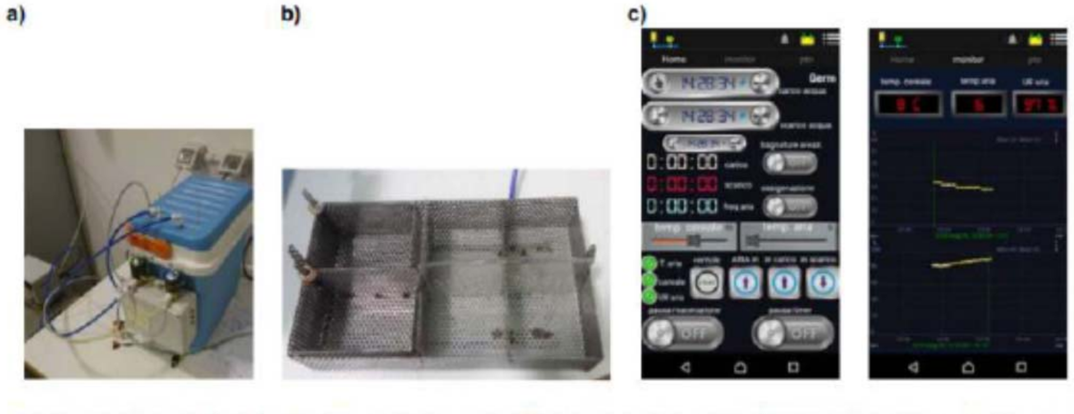

Figure 1: Pictures of the bench-top plant (a), perforated baskets (b), and Virtuino application installed on a smartphone with all the function keys monitoring the seed wetting phase (c).

The automatic control of the solenoid valves and seed temperature, data monitoring and recording were carried out using a Geekcreit® ATmega328P Nano V3 microcontroller, appropriately programmed in a C^{++} environment. The control system was remotely managed via Bluetooth connection (HC-06 transceiver) using a specific application (Virtuino) for mobile devices (Android). The following physical tests were carried out: i) *seed weight* (g), recorded as the mean weight of 50 seeds; ii) *seed volume* (in mL/seed) by transferring 50 seeds into a 100-mL cylinder, adding 50-mL of distilled water (V_W) at 20 °C, measuring the final volume of the mixture (V_{f0}), and estimating the ratio of the difference between V_{f0} and V_W and the number of seeds (n_S); iii) *seed mean radius* (R_S) by setting the displaced volume of water (V_{f0}-V_W) equal to [n_S (4/3 π R_S^3)] on the assumption that the seed initial volume was constant and the kernel had spherical conformation (Sayar et al., 2001); iv) *seed density* (in g mL^{-1}) as the ratio of seed weight and volume; v) *hydration capacity* (HC) (in g/seed) by transferring 50 seeds having an initial mass (m_0) into a 150-mL beaker, adding 50 mL of distilled water, keeping the beaker at 20 °C for 16 h, draining and weighing the seeds (m_{f16}) after removing the excess water with absorbent paper, and estimating the ratio of the difference between m_{f16} and m_o and the number of seeds used; vi) *swelling capacity* (SC) (in mL/seed) by transferring the above seeds soaked for 16 h into a 150-mL beaker, adding 50-mL of distilled water (V_W), measuring the final volume of the mixture (V_{f16}), and estimating the difference between V_{f16} and V_W divided by the number of seeds used.

Soaking trials were performed by charging about 1 kg of seeds in the 6 baskets shown in Figure 1b and submerged with deionized water kept at 12, 18, 24, 30, and 36 °C (accuracy ±0.5 °C). By removing sequentially the baskets at 0, 1, 3, 5, 16, and 24 h, the soaked kernels were quickly blotted on paper towels to remove the excess moisture adhering on the surface, weighed and analyzed for their moisture content. Thus, four seeds weighing 3.0-3.5 g were collected on the weighing plate of a Kern DAB 100-3 thermostatic scale (Kern&Sohn GmbH, Balingen, Germany), roughly divided in parts and dried till constant weight at 110 °C for as long as ~20 min. All the trials were carried out in triplicate to estimate the mean values (μ), standard deviation (sd), and coefficient of variation (CV) of data collected. The Tukey's test was applied for the statistical comparison of means at the probability level of 0.05. The hydration kinetics of several seeds is generally described using either empirical or theoretical models. The empirical model developed by Peleg (1988) has been largely used to describe the rate of water uptake of several cereals (Montanuci et al., 2013; Sopade et al., 1992) and legumes (Abu-Ghannam and McKenna, 1997; Hung et al., 2006, Pan and Tangratanavalee, 2003) undergoing hydration. Thus, the instantaneous moisture ratio M(t) can be predicted as follows:

$$M(t)=M_0+\frac{t}{k_1+k_2 t} \tag{1}$$

where t is the hydration time, M_0 the initial moisture ratio, k_1 the Peleg rate constant, and k_2 the Peleg capacity constant. The rate of water uptake (R_W) can be estimated as

$$R_W=\frac{dM}{dt}=\frac{k_1}{(k_1+k_2\,t)^2} \tag{2}$$

At t=0, the initial water uptake rate is

$$R_{W0}=\frac{1}{k_1} \tag{3}$$

As t→∞, the moisture ratio M reaches the equilibrium moisture ratio (M_e):

$$M_e=M_0+\frac{1}{k_2} \tag{4}$$

Thus, the initial water uptake rate (R_{W0}) is inversely proportional to the Peleg rate constant k_1, while the Peleg capacity constant k_2 is in connection with the equilibrium moisture content (x_{We}). By linearizing Eq. (1) as follows:

$$\frac{t}{M(t)\text{-}M_0}=k_1+k_2\,t, \tag{5}$$

it was possible to determine both k_1 and k_2 using the least squares method. Owing to the inverse relationship between R_{W0} and k_1, the dependence of k_1 on the absolute steeping temperature (T_K) was described using an Arrhenius-type relationship as follows:

$$\frac{1}{k_1}=A\exp\left(-\frac{E_a}{R\,T_K}\right) \tag{6}$$

where A is the pre-exponential nonthermal factor, E_a the activation energy, and R the universal gas constant. On the contrary, k_2, being a characteristic sorption parameter of the material examined, generally exhibits no significant temperature dependence (Montanuci et al., 2013; Sopade et al., 1992).

3. Results and Discussion

3.1. Physical properties

The physical properties of chickpea and yellow soybean samples are shown in Table 1.

The seed weight (m_S), volume (V_S), density (ρ_S), HC and SC of the chickpea samples used here were definitively greater than corresponding average values of several Sicilian (0.34±0.03 g/seed; 0.29±0.04 cm^3/seed; 1.18±0.15 g cm^{-3}; 0.036±0.09 g/seed; 0.35±0.1 cm^3/seed: Patanè et al., 2004) and Indian (Kaur et al., 2005) chickpea populations. In the circumstances, the cultivar tested here should ask for a longer cooking time (Patanè et al., 2004; Kaur et al., 2005; Waldia et al., 1996). Soybean seeds exhibit large variations in shape from almost spherical to flat and elongated, size (5-11 mm) and weight (0.12-0.18 g/seed) (Heuzé et al., 2017). In this case, the yellow soybean seeds had an average seed weight in line with that (0.175±0.002 g/seed) of seeds grown under normal climate conditions (Borrmann et al., 2009).

Table 1: Some physical properties of chickpea and yellow soybean seeds.

Physical property	**Chickpea**	**Soybean**	**Unit**
Seed weight (m_S)	0.437±0.016	0.168±0.008	g/seed
Seed volume (V_S)	0.333±0.010	0.147±0.016	cm^3/seed
Seed mean radius (R_S)	0.430±0.004	0.327±0.012	cm/seed
Seed density (ρ_S)	1.312±0.045	1.199±0.118	g cm^{-3}
Hydration capacity (HC)	0.454±0.023	0.218±0.013	g/seed
Swelling capacity (SC)	0.448±0.052	0.210±0.021	cm^3/seed

3.2. Hydration kinetics of dry pulses

Figure 2 shows the evolution of the hydration isotherms at 12-36 °C for the legumes under study. Each isotherm was characterized by an initial quick increase for t varying from 0 to 5 h. After that, there was a slower growth up to reach a saturation moisture ratio. Such a ratio tended to increase with the steeping temperature.

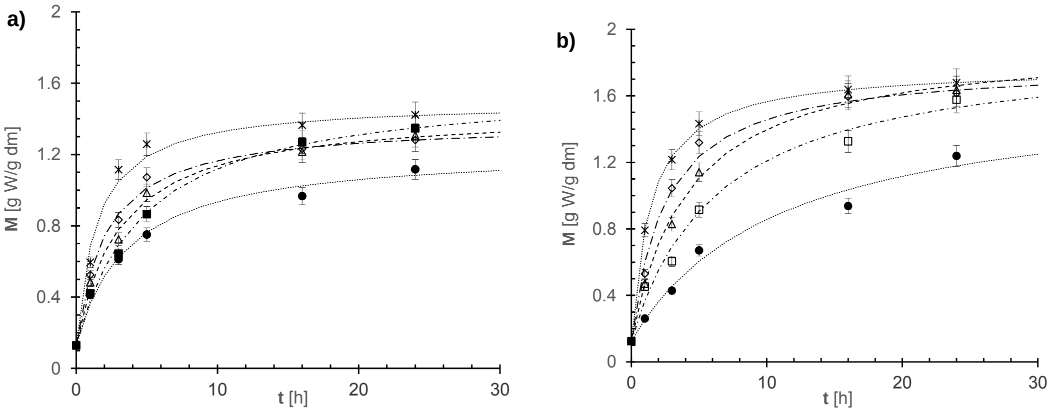

Figure 2: Time course of the experimental moisture ratio M during the steeping of chickpeas (a) and yellow soybeans (b) at different temperatures (●, 12 °C; ■, 18 °C; △, 24 °C; ◇, 30 °C; ×, 36 °C). The diverse curves were plotted using the Peleg model and the constants listed in Table 2.

Table 2: Mean values, standard deviations, and coefficients of determinations (r^2) of the Peleg constants k_1 and k_2 characterizing the hydration kinetics of the chickpea and yellow soybean samples at different temperatures.

Temperature [°C]	**k_1** [h g dm/g W]	**k_2** [g dm/g W]	**r^2**	**k_1** [h g dm/g W]	**k_2** [g dm/g W]	**r^2**
	Chickpea			***Yellow Soybean***		
12	3.3±0.6 ^{a}	0.91±0.05 ^{a}	0.991	7.1±1.1 ^{a}	0.65±0.08 ^{a}	0.952
18	3.3±0.3 ^{a}	0.68±0.02 ^{b}	0.997	3.6±0.7 ^{b}	0.56±0.05 ^{a}	0.976
24	2.3±0.3 ^{b}	0.76±0.02 ^{c}	0.998	2.3±0.2 ^{c}	0.55±0.02 ^{a}	0.997
30	1.7±0.2 ^{c}	0.80±0.01 ^{d}	0.999	1.5±0.2 ^{d}	0.60±0.02 ^{a}	0.998
36	1.1±0.2 ^{d}	0.73±0.02 ^{e}	0.999	0.87±0.04 ^{e}	0.607±0.003 ^{a}	1.000

- Different lowercase letters indicate statistically significant difference among k_1 or k_2 values at different steeping temperatures at the probability level of 0.05.

For both legumes, the experimental moisture ratios were fitted using Eq. (5), and the Peleg constants k_1 and k_2 were estimated using the least squares method, as listed in Table 2. The coefficients of determination (r^2) for the five temperatures under examination ranged from 0.991 to 0.999 for chickpea samples and from 0.952 to 1.000 for yellow soybean ones. Thus, Eq. (1) appeared to be an appropriate model for reconstructing the water absorption of both legumes during steeping. In these tests, the Peleg rate constant k_1, which is the reciprocal of the initial water uptake rate (R_{W0}) according to Eq. (3), decreased with increasing temperature in the range of 12-36 °C. By resorting to Eq. (6), the natural logarithm of the reciprocal of k_1 was linearly related to the reciprocal of the absolute steeping temperature (T_K), and the pre-exponential factor (A) and activation energy of the hydration process (E_a) were estimated using the least squares method (Table 3).

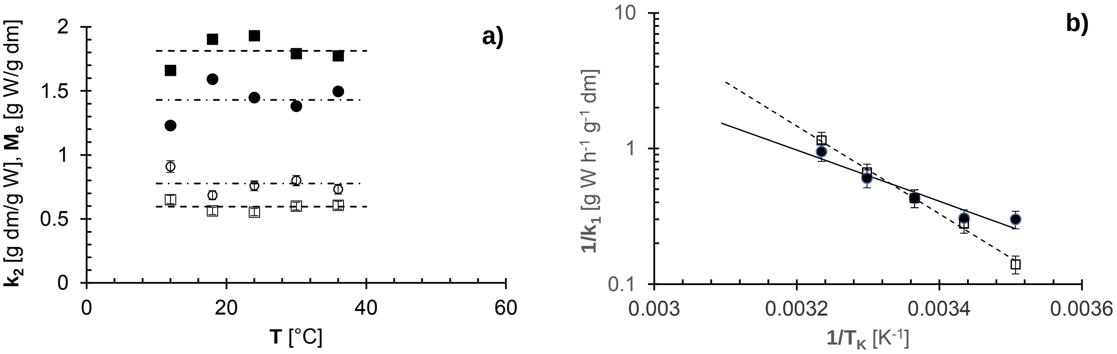

Figure 3: Effect of steeping temperature on (a) the Peleg capacity constant (k_2: ○, ☐), and equilibrium moisture ratio (M_e: ●, ■), and (b) the reciprocal of the Peleg rate constant (k_2: ●, ☐) characterizing the steeping behavior of the chickpea (●, ○) and yellow soybean (■, ☐) samples. The continuous and broken lines were plotted using Eq. (6) with the constants listed in Table 3.

Table 3: Mean values and standard deviations of the pre-exponential factor (A) and activation energy (E_a), coefficient of determination (r^2), Peleg capacity constant (k_2), equilibrium moisture ratio (M_e) and weight fraction (x_{We}) characterizing the hydration kinetics of chickpea and yellow soybean samples.

Legume	**Chickpea**	**Yellow Soybean**	
Parameter	**Value**	**Value**	**Unit**
A	13.86±2.38	24.3±1.1	h^{-1} g W/g dm
E_a	36.1±5.9	60.0±2.7	kJ mol^{-1}
r^2	0.926	0.994	-
k_2	0.78±0.09	0.60±0.04	g dm/g W
M_e	1.43±0.14	1.81±0.11	g W/g dm
x_{We}	0.59±0.02	0.64±0.01	g/g

According to Sayar et al. (2001) and Sopade et al. (1992), the rate of water uptake in legumes is controlled by the diffusion phenomenon. In this work, the sensitivity of chickpea samples to steeping temperature was characterized by an activation energy of the same order of magnitude (20-50 kJ mol^{-1}) of spring and winter chickpeas at lower temperatures (20-55 °C) than the starch gelatinization threshold (Sayar et al., 2001). The lower the value of E_a the more thermally stable the seeds are, their hydration behavior being less affected by temperature. Yellow soybean samples presented a greater E_a value and, according to Eq. (3), a slower initial water uptake rate than chickpea ones especially at $T<24$ °C (Table 3). The Peleg capacity constant (k_2), being related to the saturation moisture of the kernel examined, displayed no significant temperature dependence in the case of yellow soybean samples at a confidence level of 95%. In the case of chickpea ones, k_2 ranged from 0.68 to 0.91 g dry matter (dm) per g of water (W), but it was for the sake of simplicity regarded as constant, as shown in Figure 3a. By using Eq. (4), the estimated value of the equilibrium moisture ratio (M_e) or weight fraction (x_{We}) was equal to 1.4 g W/g dm or 59% (w/w) for chickpea samples and to 1.81 g W/g dm or 64% (w/w) for yellow soybean ones, respectively (Table 3).

The hydration kinetics of both the legumes examined here appeared to be different from that of barley at 12-15 °C (Brookes et al., 1976), where three different soaking phases are generally accounted for. Phase 1, lasting from 6 to 10 h, is characterized by quick water uptake and leads to 60 % of the overall water absorbed. Phase 2 is associated to a very slow water absorption and may be prolonged for as long as 10 h. Phase 3 exhibits a faster water uptake, generally stopped by the so-called *steep-ripe plateau*, after which the semipermeable membrane of the grain breaks down and germination becomes visible. Generally, such a process takes place after 46-h wetting at a moisture content of 41-52 % (w/w) and temperatures of 10-20 °C (Brookes et al., 1976). Because of the prolonged period of steeping, as the moisture content of barley seeds was greater than 32 % (w/w) a certain metabolic activity of the germ takes place. To assure optimum conditions for the embryo activity, it is essential to provide enough oxygen and remove the CO_2 formed. In this work, the hydration kinetics of both seeds was faster. Their moisture weight fraction increased to ~50% (w/w) just after 5-h soaking at $T \geq 24$ °C (Figure 2) reaching about 78-85% of the equilibrium weight fraction (x_{We}). In the circumstances, such a relatively short steeping process appeared to be sufficient to initiate the germination phase without the aid of any aeration system or air rest phase.

3. Conclusions

The hydration kinetics of chickpea and yellow soybean kernels at 12-36 °C was adequately reconstructed via the well-known Peleg model. For both seeds, their equilibrium moisture ratio (M_e) appeared to be independent of temperature. The water absorption rate for these legumes resulted to be quicker than that detected during traditional steeping of barley. A 5-h soaking at $T \geq 24$ °C allowing both seeds to be rehydrated to a moisture content of ~50% (w/w) was retained as sufficient for activating the metabolic processes of germination. Further work is needed to assess the kinetics and yields of seed germination and main anti-nutritional factor removal.

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