Thèse

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Procédés de production et influence des propriétés fonctionnelles des poudres de calices d'*Hibiscus sabdariffa* et de leurs fractions sur la formulation de boisson

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13 Décembre 2022

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AFM	Atomic Force Microscopy
ANOVA	One-way analysis of variance
B10	Grinding at 10 000 rpm
B12	Grinding at 12 000 rpm
B14	Grinding at 14 000 rpm
C	Coarse
CBD	Compressed bulk density
ESI	Electrospray Ionization
F	fine
FAO	Food and Agriculture Organization
HS	Hibiscus
HSD	Honestly Significant Difference
MA	Mass area
MS	Mass spectrometry
MS/MS	Two stage mass spectrometry
OD	Oven-Drying
ODD	Objectifs de développement durable
PAOT	Pouvoir Antioxydant Total
PCA	Principal Component Analysis
PDA	Photodiode Array Detector
SD	Solar-drying or sun-drying
SDGs	Sustainable Development Goals
UHPLC	Ultra high-performance liquid chromatography
UV	Ultra Violet
W	Whole
Φ_1	Phase 1 (during reconstituted powder dispersion)
Φ_2	Phase 2 (during reconstituted powder dispersion)

Grandeurs :

AA	Antioxidant activity
[Anthocyanin]	Anthocyanin concentration
A	Absorbance
a*	Red-green balance (color parameter)
ABTS	2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid
AR	Aspect Ratio
a_w	Water Activity
b*	Yellow-blue balance (color parameter)
BI	Browning Index

C*	Chroma value (color parameter)
CI	Color Intensity
D_{10}	Diameter for which 10 % of sample particles are smaller
D_{50}	Diameter for which 50 % of sample particles are smaller
D_{90}	Diameter for which 90 % of sample particles are smaller
D_{Feret}	Distance between two parallel tangents to the particle at an arbitrary angle
El	Elongation
$E_{product\ 0}$	Electrochemical potential of the reaction medium immediately after powder addition
$E_{product\ 10}$	Electrochemical potential of the reaction medium 10 min after powder addition
EQPC	Equivalent projected area of a circle
Fb	Fiber proportion
F_d	Dilution factor
ff	Flow factor
IM	Insoluble Material
H*	Hue angle (color parameter)
l	Length of the spectrophotometer cell
L*	Lightness (color parameter)
M	Molarity
m/z	Mass out of the charge number of ions
MM	Molar mass
P_{EQPC}	Perimeter of equivalent projected area of a circle
P_{real}	Real perimeter
R	Correlation coefficient
RH	Relative Humidity
RT	Retention time
S	Sphericity
Span	Width of the size distribution relatively to the median value of powder
T0	Sun-drying
T1	Sun-drying + 1 h oven-drying
$t_{1/2}$	Half-time
T2	Sun-drying + 2 h oven-drying
Tg	Glass transition temperature
Y_N	Normalized value
Y	Value to be normalized
Y_{max}	Maximum value of y
Y_{min}	Minimum value of y
σ_1	Major principal stress
σ_c	Unconfined yield stress

Unités :

AU	Arbitrary Unit
g	Gram
× g	Gravity (times gravity)
h	Heure
J	Joule
kPa	kilopascal
kV	Kilovolt
mg	Milligram
Min	Minute
mL	Milliliter
mm	Millimeter
nm	Nanometer
Pa	Pascal
Rpm	Rotation per minute
s	Seconde
µS	Microsiemens

Symboles :

°C	Celsius degree
%	Percentage
µL	Microliter
Ɛ	Molar extinction coefficient
ΔE	Total color change (the calcul includes L*, a*, and b*)
θ	Angle between liquid and solid surfaces

Chapitre 1 : Introduction

1.1. Contexte

« Les fruits et légumes sont le fondement d’une alimentation saine et variée. Ils apportent à l’organisme une abondance de nutriments, renforcent le système immunitaire et contribuent à réduire les risques de maladies. Et pourtant, malgré ces avantages considérables, nous n’en consommons pas assez. » a déclaré António Guterres, secrétaire général de l’ONU (Organisation des Nation Unies) en 2021, année internationale des fruits et légumes pour ainsi insister sur l’enjeu de la consommation des végétaux. La FAO (Organisation des Nations Unies pour l’Alimentation et l’Agriculture) définit l’ensemble « fruits et légumes » comme étant toutes les parties comestibles (structures porte-graines, fleurs, bourgeons, feuilles, tiges, pousses, racines, etc.) des végétaux cultivés ou récoltés (FAO, 2021). L’importance d’une alimentation saine pour le consommateur suscite de nombreux programmes de vulgarisation et sensibilisation à la consommation des fruits et légumes frais ou très peu transformés (ayant subi des opérations telles que le lavage, l’épluchage, le tranchage, le conditionnement, la congélation, le séchage...) appelés « minimally processed food ». La FAO ambitionne de renforcer la production durable d’aliments sains grâce à l’innovation et aux technologies, ainsi qu’à réduire les pertes alimentaires. Cette sensibilisation renvoie également à des enjeux sociétaux majeurs tels que l'environnement, la santé et la sécurité alimentaire. Consommer régulièrement des produits végétaux permet de limiter la faim, de maintenir un mode de vie sain, de prévenir les maladies et de limiter le gaspillage alimentaire de ces produits très périssables. Une consommation variée de fruits et légumes favorise la polyculture et par conséquent la biodiversité pour un environnement durable. Cette visée est en concordance avec les Objectifs de Développement Durable (ODD) liés aux végétaux qui reposent sur :

- Les effets bénéfiques des fruits et légumes sur la santé ;
- La sensibilisation à une alimentation diversifiée et saine ;
- La durabilité pour améliorer la disponibilité, la sécurité sanitaire et l’accessibilité financière des fruits et légumes ainsi que l’accès équitable à ceux-ci, favorisant par conséquent la durabilité économique, sociale et environnementale ;
- La valorisation du rôle des exploitants familiaux, car la culture des fruits et légumes génère des revenus, renforce les moyens d’existence, améliore la sécurité alimentaire, et accentue la résilience grâce à la gestion durable des ressources locales et à la promotion de l’agro-biodiversité ;

- La réduction des pertes alimentaires.

La consommation régulière des végétaux frais permettrait de limiter les pertes alimentaires mais cette éventualité reste théorique face à la répartition géographique inégale des cultures. Comme alternative, des recours à la transformation des produits agricoles, ou l'utilisation de certains conservateurs permettent d'assurer la conservation des fruits et légumes et leur transport des régions productrices vers les zones non-productrices. Cependant les transformations et l'utilisation de conservateurs non naturels ne sont pas sans impacts sur la santé du consommateur car peuvent réduire considérablement la valeur nutritionnelle des produits et être à l'origine de certaines maladies. Cependant une transformation minimale, par exemple un séchage contrôlé, peut assurer à la fois une longue durée de conservation (pour les aliments riches en eau) et préserver une grande partie des propriétés inhérentes au produit (physiques, chimiques, nutritionnelles, et organoleptiques).

Dans la catégorie des fruits et légumes, sont classés les calices d'*Hibiscus sabdariffa* (hibiscus), des végétaux en vogue, du fait de leur coloration rouge attrayante et de leur potentiel impact bénéfique sur la santé notamment en lien avec leur richesse en molécules antioxydantes : anthocyanes et polyphénols. L'*Hibiscus sabdariffa* présente en plus un potentiel économique car il constitue un marché important. Par exemple, l'exportation des calices secs du Sénégal vers l'Europe et les États-Unis était estimée à 1300 - 3300 $€{\cdot}t^{-1}$ et augmentait d'année en année (Cisse, 2010), la France étant en 2020 le 4^e^ pays importateur d'hibiscus ("Importations mondiales et principaux pays importateurs de *Hibiscus sabdariffa*," 2020).

Force est de constater que les calices d'hibiscus sont consommés et commercialisés à travers le monde, à l'état frais (dans les villes productrices), mais majoritairement à l'état sec. La nécessité de l'opération de séchage découle du caractère très périssable des calices frais. Leur forte teneur en eau (80 – 90 g d'eau / 100 g de produit) amplifie la sensibilité au développement microbien en particulier dans les environnements chauds et humides. La conservation de la qualité des calices frais requiert donc un déploiement de moyens financiers colossaux pour assurer la réfrigération pendant la durée d'acheminement des calices frais d'une ville productrice vers une autre ville locale (zones tropicale et subtropicale), et plus encore lors des exportations vers les pays non producteurs. La fanaison des fleurs, le risque de dégradation des biomolécules, de contamination microbienne, le cout élevé du stockage des calices frais, et le risque de pertes alimentaires ont incité les producteurs à recourir au séchage après la récolte. Le séchage est une opération unitaire qui permet d'accroitre la durée de conservation du produit et en complément, il permet de faciliter les échanges mondiaux car cette opération aboutit à une réduction du volume et du poids des récoltes, et par conséquent à une diminution des coûts de transport. Cette opération unitaire est donc pertinente et essentielle dans la chaine de production. Néanmoins, le séchage, en fonction de la méthode employée, peut réduire la qualité nutritionnelle du produit en dégradant les biomolécules thermolabiles notamment les anthocyanes. Contrôler et optimiser les paramètres de cette opération (type de séchage, température, durée) limiterait ces pertes en biomolécules. Actuellement, le type de séchage le plus appliqué à l'hibiscus est le séchage solaire qui, cependant, n'est pas entièrement contrôlable étant donné que ce séchage est dépendant des conditions météorologiques : durée d'ensoleillement, température, vitesse de l'air, humidité de l'air. Les calices d'hibiscus secs sont ensuite généralement consommés sous forme de boisson chaudes ou froides après une étape d'infusion et sont également transformés en gelée, confiture, confiseries ou encore, utilisés comme colorants alimentaires et cosmétiques. L'hibiscus est également exploité en médecine et pharmacie pour ses anthocyanes car ils sont comptés parmi les antioxydants naturels utilisés pour remplacer progressivement les antioxydants synthétiques (Vilas-Boas et al., 2021).

La boisson d'hibiscus étant très prisée, elle est vendue prête à l'emploi pour faciliter l'utilisation par le consommateur. Seulement, ses biomolécules sont instables en milieu aqueux et se dégradent pendant le stockage aboutissant à une décoloration du produit et une perte du pouvoir antioxydant. Ainsi, une alternative est la production de poudre d'hibiscus afin d'obtenir un produit présentant une faible activité de l'eau et prêt à être infusé/reconstitué par le consommateur.

Les méthodes de production de poudre de l'hibiscus incluent généralement la préparation d'un extrait de calices par infusion puis sa conversion en poudre par séchage par atomisation. Il est également possible de produire des poudres sans passer par une étape d'extraction mais en réalisant un séchage des calices suivi d'un broyage. Le broyage peut être mené de façon à obtenir de très fines particules (de l'ordre du micromètre), on parle alors de micronisation. Cette méthode est de plus en plus, employée pour convertir les végétaux en poudres tels que le thé vert, le jiaogulan, la coriandre, le moringa, le mangoustan, le riz noir germé, le curcuma, la spiruline (Elisabeth, 2015) et dans une moindre mesure l'hibiscus. Cependant l'étude et la compréhension de l'impact de chaque opération unitaire de la micronisation restent encore à approfondir pour une meilleure valorisation des produits et de leurs propriétés fonctionnelles. Actuellement, le séchage couplé au broyage des calices d'hibiscus est principalement considéré comme une étape de préparation des échantillons en vue de leurs analyses biochimiques, physico-chimiques, et microbiologiques, pourtant, l'obtention de poudres par un tel procédé dans des conditions contrôlées pourrait permettre la production de produits peu transformés répondant aux objectifs mentionnés par la FAO.

1.2. Objectifs de la thèse

Le présent projet vise à proposer un procédé de stabilisation pour préserver au mieux les vertus et propriétés initiales des calices d'*Hibiscus sabdariffa* afin d'en faciliter l'accessibilité et l'utilisation. Dans cette optique, le projet a été axé sur l'étude du séchage couplé au broyage, pour produire des poudres d'*Hibiscus sabdariffa* en répondant aux questions scientifiques suivantes qui constituent le fil conducteur de ce travail :

- Comment les paramètres opératoires du séchage solaire et du broyage impactent-ils les propriétés physicochimiques et fonctionnelles des poudres ?
- Quel est le mécanisme de reconstitution des poudres d'hibiscus et quelle est la cinétique de libération des biomolécules associée ?
- Quel est l'impact des procédés de production de poudres sur l'activité des biomolécules ?
- Comment le passage d'un séchage solaire à un étuvage couplé au broyage et au tamisage permet d'orienter les propriétés fonctionnelles des poudres ?

Ces questions scientifiques permettent d'organiser le présent travail en trois grandes parties illustrées sur la **Figure 1** :

- Interactions entre les paramètres de procédés et les propriétés des poudres *d'Hibiscus sabdariffa* ;
- Compréhension du mécanisme de reconstitution des poudres ;
- Améliorations des propriétés fonctionnelles et de l'activité antioxydante des poudres.

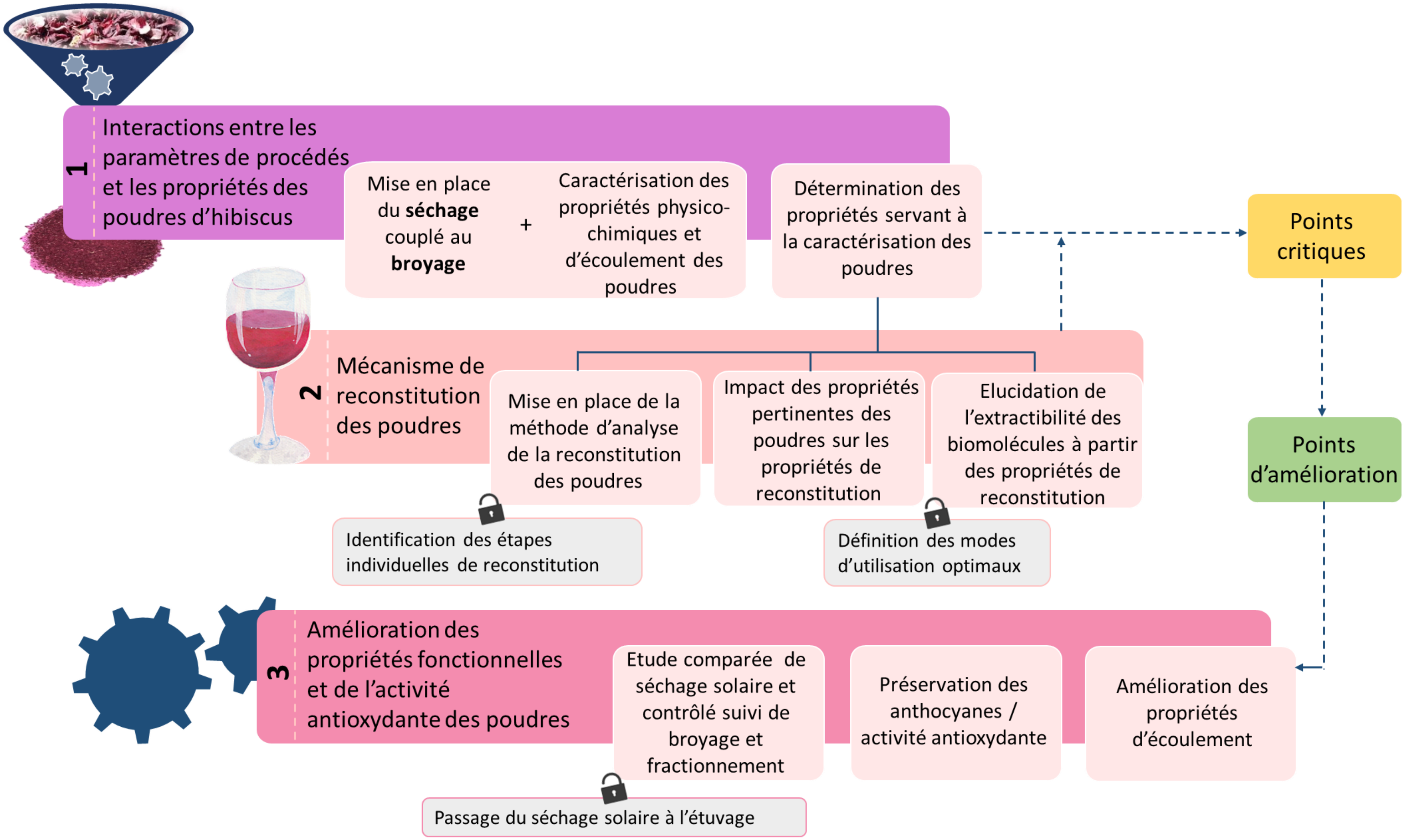


Figure 1: Organisation de la thèse en trois parties principales.

1.3. Valorisation scientifique

- **Publications**

C.U. M'BE, J. SCHER, J. PETIT, N.G. AMANI, J. BURGAIN, Relationship Between Drying and Grinding Parameters and Physicochemical Properties of *Hibiscus sabdariffa* Calyx Powders, *Particulate Science and Technology*, Taylor & Francis, 2022, pp. 1–10, doi:10.1080/02726351.2022.2032508.

C.U. M'BE, J. SCHER, J. PETIT, C. PARIS N.G. AMANI, J. BURGAIN, Reconstitution mechanism of *Hibiscus sabdariffa* powders and evaluation of antioxidant activity of resulting aqueous solutions (soumise au journal Powder Technology).

C.U. M'BE, J. SCHER, J. PETIT, C. PARIS N.G. AMANI, J. BURGAIN, Impact of powder processing and physicochemistry of *Hibiscus sabdariffa* calyxes on antioxidant activity: a review (soumise au journal Drying Technology).

C.U. M'BE, J. SCHER, I. DJIOUA, N.G. AMANI, J. BURGAIN, Valorisation des calices d'*Hibiscus sabdariffa* riches en anthocyanes pour la formulation de boissons (soumise à la revue IAA – Industries Agro-Alimentaires).

- **Communications orales**

C.U. M'BE, J. SCHER, J. PETIT, NG.G. AMANI & J. BURGAIN, Propriétés fonctionnelles des poudres de calices d'*Hibiscus sabdariffa* et de leurs fractions. Université Nangui Abrogoua, Côte d'Ivoire, Abidjan, 10 Décembre 2020.

C.U. M'BE, J. SCHER, J. PETIT, NG.G. AMANI & J. BURGAIN, Impact des paramètres de procédé de séchage et de broyage sur les propriétés d'écoulement des poudres de calices d'*Hibiscus sabdariffa.* Conférence Sciences et technologie des poudres matériaux frittés 2021, France, Saint Etienne, 7 juillet 2021.

- Communications par affiche

C.U. M'BE, J. SCHER, J. PETIT, NG.G. AMANI & J. BURGAIN, Impact of processing parameters on the functional and physicochemical properties of *Hibiscus sabdariffa* calyx powders, Séminaire de l'école doctorale Sciences et Ingénierie des Ressources Naturelles, France, Nancy.

C.U. M'BE, J. SCHER, J. PETIT, C. PARIS, N.G. AMANI, J. BURGAIN, Valorization of whole calyxes of *Hibiscus sabdariffa* by production of powders with high nutritional value, 5th Food Structure and Functionality Symposium, Ireland, Cork.

1.4. Projet Nagoya

Les calices d'*Hibiscus sabdariffa* utilisés pour l'étude sont fournis par le biais de l'Université Nangui Abrogoua (Côte d'Ivoire). Dans un but de protéger les ressources génétiques échangées (importation / exportation) entre les pays, ainsi que les connaissances traditionnelles associées à ces ressources, un protocole nommé Nagoya a été mis en place. La France et la Côte d'Ivoire étant des pays signataires du projet Nagoya, les démarches ont été entamées en partenariat avec le service valorisation innovation de l'UL et l'université Nangui Abrogoua, afin de respecter les réglementations portant sur l'exportation et la recherche scientifique de l'*Hibiscus sabdariffa*.

Chapitre 2 : State of the art

Introduction à l'état de l'art

L'*Hibiscus sabdariffa* (hibiscus) est une plante tropicale aux calices rouges dont la coloration, les minéraux, les vitamines, les anthocyanes, la teneur en phénols et l'activité antioxydante la rendent intéressante pour les consommateurs, d'un point de vue nutritionnel et médicinal. Les calices de cette plante saisonnière sont riches en eau, d'où la nécessité de les stabiliser par séchage. Ce traitement, avec en complément la transformation en poudre, facilite l'accessibilité de la plante et donc de ses bienfaits médicinaux à travers le monde entier, mais également améliore la disponibilité des biomolécules. Pour cela, il est indispensable que les opérations unitaires soient conduites de façon à ce qu'elles n'engendrent pas de dégradation des composants de l'hibiscus. L'état de l'art de ce travail de thèse aborde les connaissances actuelles sur l'hibiscus comprenant la composition, les utilisations, les bienfaits médicinaux, puis développe les méthodes de stabilisation généralement appliquées, notamment le séchage (séchage solaire, lyophilisation, étuvage), l'extraction, et les méthodes de conversion en poudre (séchage par pulvérisation, broyage). En complément, les avantages et inconvénients de chaque traitement et leurs influences sur la qualité physique et chimique des produits sont présentés.

Cet état de l'art a été soumis au journal « Drying Technology » en tant que revue bibliographique portant le titre suivant : Impact of powder processing and physicochemistry of *Hibiscus sabdariffa* calyxes on antioxidant activity: a review.

2.1. Consumer preferences for food products

Consumers are progressively turning to natural products, such as plant products, for several reasons, especially for their nutritional quality and their health properties. These characteristics are attributed to the vegetable richness in vitamins, minerals, and some compounds essential to the functioning of the human body (Food and Agriculture Organization (FAO), 2001). People preference for natural products is a way to guarantee their quality food since the health effect of synthetic food ingredients have long been controversial. The medicinal benefits of plant products are partly attributed to their natural richness in antioxidant compounds. As a result, attention is also being paid to the substitution of synthetic antioxidant products with natural antioxidants (Vilas-Boas et al., 2021) or antioxidant-rich natural products, even though the current market offers mostly synthetic minerals, vitamins, and antioxidants. The calyx of the *Hibiscus sabdariffa* fits well in this context because this plant contains natural minerals, biomolecules such as polyphenols, flavonoids, and specifically anthocyanins (Cisse, 2010; Navidad-Murrieta et al., 2020; Sinela, 2016), hence the name "functional foods". Functional foods are "those foods that encompass potentially healthful products, including any modified food or ingredient that may provide a health benefit beyond the traditional nutrients it contains" as defined by the Institute of Medicine in Washington (Bagchi, 2016). The definition given by Hasler 2002 and Falk 2004 states that functional food contains biologically active components, vitamins, phenolic acids, flavonoids, anthocyanins that give the products the health benefits (Cid-Ortega and Guerrero-Beltran, 2014; Falk, 2004; Hasler, 2002). The interest in functional food is growing (Bagchi, 2016; Loizzo and Silva, 2021), Americans and Japanese are leading the market, followed by Asians and Europeans (Bagchi, 2016). On the other side, attention must be paid to organoleptic criteria since Bagchi 2016 in his book, points out the non-acceptability of certain functional foods because the sensory properties and the marketing criteria do not captivate the consumer. *Hibiscus sabdariffa* (hibiscus) calyxes meet these conditions by their red color and their sour-fruity taste (M. J. P. Monteiro et al., 2019), which are the first criteria for the choice and acceptability (Plotto et al., 2004a) of the final product by consumers, an advantage for this plant. In addition, FAO has declared 2021 as the year of plant products, including plants that are consumed fresh or that are minimally processed (washing, peeling, slicing, packaging, freezing, drying...) like fresh or dried *Hibiscus sabdariffa* calyxes. This sensitization relates to major societal issues such as the environment, health, and food safety. One of the most important issues is the overall health benefits that consumers get from using plants. Polyphenols and anthocyanins of plants products promote an important antioxidant activity that is exploited in the health community. These biomolecules wealth attributes many health benefits to *Hibiscus sabdariffa* calyxes, and makes them highly coveted products. After studying the biomolecules and identifying their health benefits, the evaluation of the economic and health potential of these calyxes has prompted researchers to develop processes in the medical field to extract as many molecules of interest as possible to reuse them to treat diseases. However, the limitation of processing remains a great challenge because it is an important criterion for the preservation of the initial properties (antioxidant) of products to take advantage of their health benefits and nutritional quality. This is not always the case in the food industry, where dried calyxes are used to product extract liquid before being transformed into powder (Eroğlu et al., 2018; Gonzalez-Palomares et al., 2009). The extraction step can limit the protection of the anthocyanins, whereas if the powder is obtained by grinding, a more favorable environment for these biomolecules could be maintained. The processing of *Hibiscus sabdariffa* such as drying is also conducted to obtain final products that will ensure bioavailability, world export, and conservation over a long period. Indeed, *Hibiscus sabdariffa* is a tropical annual plant, whose richness in the water of the calyxes, limits the use by short shelf-life and inaccessibility to the product, particularly by the non-producing regions (Europe, United States). For this reason and to meet the health and organoleptic expectations of consumers, while facilitating the bioavailability of molecules, the calyxes have been subjected to many process studies like drying, extraction, spray-drying.

2.2. *Hibiscus sabdariffa* plant

2.2.1. Production, growing and culture

Hibiscus sabdariffa is a herbaceous plant of the Malvaceae family (Da-Costa-Rocha et al., 2014) cultivated in many tropical and subtropical countries (Figure 2) (Ahmed et al., 2019; Chumsri et al., 2008; Cisse, 2010; Da-Costa-Rocha et al., 2014; Sinela, 2016). The leaves of this annual plant are alternate, with solitary flowers. From this Malvaceae family, two botanical types of *Hibiscus sabdariffa* are distinguished: H. sabdariffa variety sabdariffa and H. sabdariffa variety altissima. The latter rich in fiber is used as a substitute for jute, coarse sacking (Da-Costa-Rocha et al., 2014). The stems of the sabdariffa variety are employed for cattle feeding or textile and the edible parts are leaves, seeds, and calyxes (Cisse, 2010). Two types of *Hibiscus sabdariffa* calyxes exist, red and the other white, with similar compound content except for anthocyanins. However, there may be some variations in proximate composition between the same varieties due to geographical location, harvesting methods, and analysis methods (Ahmed et al., 2019). Many varieties of *Hibiscus sabdariffa* with red calyxes exist. For example, Vimto (the most appreciated), Koor, CLT 92, Thaï, Burkinabe, Yoump, Violette are all Senegalese varieties with specific features (Cisse, 2010). The world's best crops come from Sudan, the most important African producer (Plotto et al., 2004a).

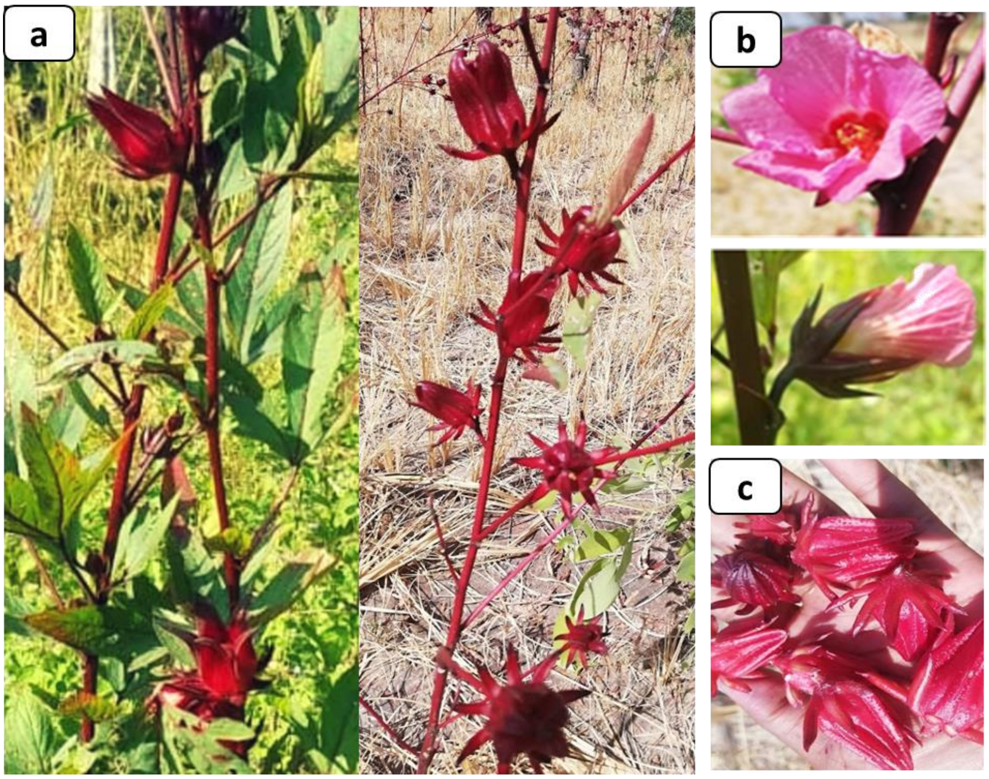


Figure 2: *Hibiscus sabdariffa*, a – plant, b – flowers, c – calyx.

Different vernacular names are given to the red calyxes such as Jamaica flowers in Central America, Krachiap Daeng in Thailand, sorrel in Guinea, bissap in Senegal and Ivory Coast, karkade in north Africa, ngai-ngai in Central Africa, Folere in Cameroun (Ahmed et al., 2019; Chumsri et al., 2008; Cisse, 2010; Ramírez-Rodrigues et al., 2011; Sinela, 2016).

Hibiscus plants growth requirements are: a minimum temperature of 20 °C, drained soils, although they can grow in poor soil, and reach maturity after 4 to 8 months (Cisse, 2010; Da-Costa-Rocha et al., 2014; M. J. P. Monteiro et al., 2019; Plotto et al., 2004b). The growing phase may be divided into 4 steps. The first step is sowing which is done during the rainy season from June to July in Ivory Coast or July to August in Senegal. Secondly, the vegetative development including the stem, branches, and leaves growth, begins. Simultaneously, petals grow, unfurl and fruits develop, highlighting the beginning of the third step of calyx development. During this period, petals close until their abscission (Figure 3), which marks the ripening of fruits and calyxes, and the beginning of the last step that is harvesting. The tender and fleshy red calyxes are collected 2 or 3 weeks after flowering by cutting them at their base (Cisse, 2010). Calyxes harvesting occurs between November and January in Senegal and Ivory Coast but it depends on the rainy season. Calyxes are then shelled, generally sun-dried, packaged, and stored.

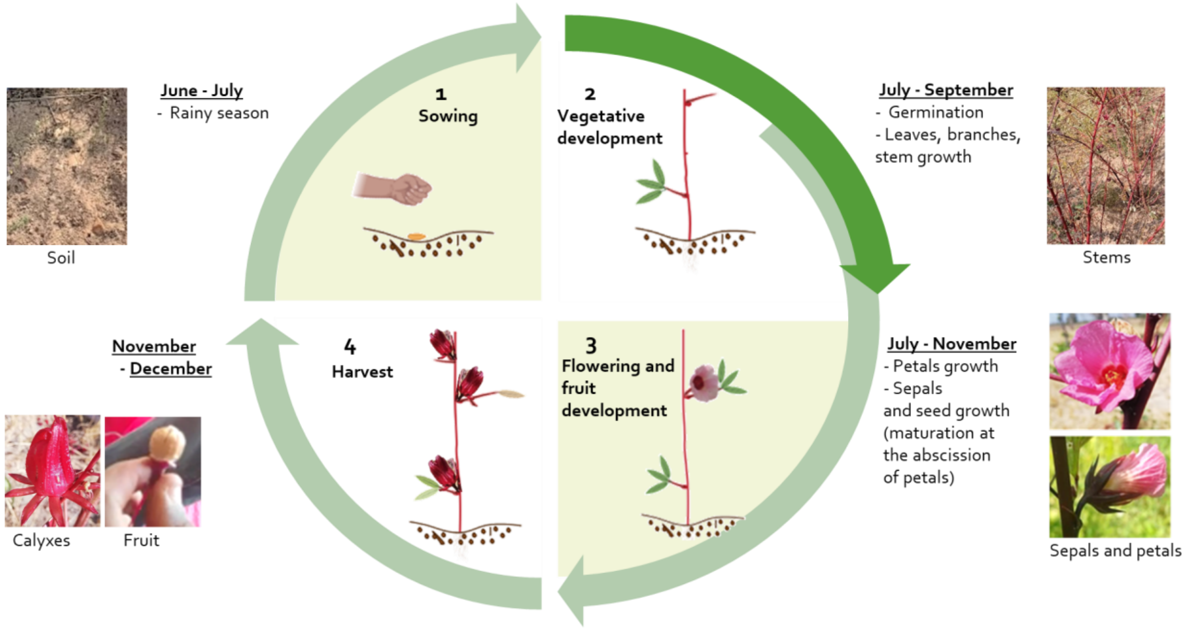
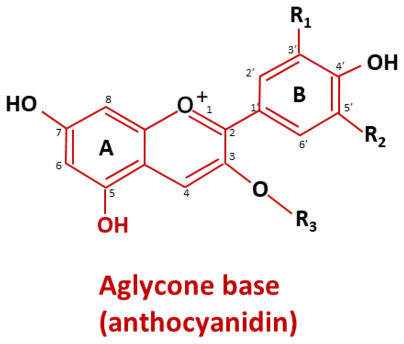


Figure 3: The growth stages of *Hibiscus sabdariffa* plant.

2.2.2. Chemical composition

The composition and physicochemical properties of *Hibiscus sabdariffa* calyxes vary according to varieties, origin, and culture method. Fresh calyxes are sources of water, proteins, fibers, and carbohydrates (Cisse, 2010). Studies reported that fresh calyxes roughly contain 80 to 90 g / 100 g water content (Cisse, 2010; Gonzalez-Palomares et al., 2009), 0.9 – 17.9 g / 100 g protein, 0.1 - 3.9 g / 100 g lipid, 2.3 - 12 g / 100 g fiber, 3.3 - 12.3 g / 100 g carbohydrates with 40 % of glucose (Cisse, 2010; Da-Costa-Rocha et al., 2014). In addition, hibiscus calyxes are a source of mineral elements (K, Ca, Mg, Fe, Mn, Zn) and organic acids (malic, citric, stearic, tartaric, ascorbic, succinic, oxalic acids) (Cisse, 2010). Succinic and oxalic acids represent together 76 % of all organic acids (Abu-Tarboush et al., 1997; Ali et al., 2005; Cisse, 2010; Da-Costa-Rocha et al., 2014; Dafallah and Al-Mustafa, 2012; Wong et al., 2002). The average ascorbic acid content is about 72 mg / 100 g (Cisse, 2010) that is higher than orange juice ranging from 49 to 54 mg / 100 g (Kabasakalis, 2000; Stinco et al., 2015) and 2.5 and 3 times higher compared to that of blackcurrant and grapes respectively (Tham et al., 2018; Yaacob, 2006). This wide range of acids imparts *Hibiscus sabdariffa* calyxes an acidic pH < 3 (Ahmed et al., 2019; Cisse, 2010; Sánchez-Feria et al., 2021) and favors the dissolution of hibiscus minerals (Adewusi et al., 1999; Jung et al., 2013). The red color of hibiscus calyxes is due to the presence of the anthocyanin molecule that is made up of two parts: a carbohydrate part linked to an aglycone base called anthocyanidin which has two aromatic cycles A and B, and an oxygen-containing heterocycle (Figure 4). The anthocyanin content (150 – 1500 mg / 100 g) (Cisse, 2010; Da-Costa-Rocha et al., 2014) can be about three times higher than in black grapes (50 - 300 mg / 100 g) (Chira et al., 2008). Two major anthocyanins (Figure 4) have been identified, delphinidin-3-sambubioside or delphinidin-3-xylosylglucoside and cyanidin-3-sambubioside or cyanidin-3-xylosylglucoside, respectively 71 and 29 % of all anthocyanins and two minor, delphinidin-3-glucoside and cyanidin-3-glucoside (Cisse, 2010; Pérez-Torres et al., 2013; Sinela, 2016; Tham et al., 2018; Wong et al., 2002). In addition to its red color, the strong antioxidant power of anthocyanin increases the interest in exploiting these molecules. Indeed, anthocyanins are the main molecules responsible of the antioxidant activity of the hibiscus calyxes (Tsai et al., 2002). Moreover, *Hibiscus sabdariffa* calyxes contain phenolic compounds including protocatechuic acid and catechin a type of natural phenol presenting antioxidant properties (Da-Costa-Rocha et al., 2014; Tham et al., 2018).



Cyanidin : R1 = OH ; R2= H ; R3 = Sambubioside

Delphinidin : R1 = OH ; R2 = OH ; R3 = Sambubioside

Figure 4: Chemical structure of major anthocyanins in *Hibiscus sabdariffa* calyx.

This calyx composition, its high anthocyanin content, antioxidant activity, and its sour-fruity taste makes the *Hibiscus sabdariffa* calyx a very coveted plant part in several fields of activity such as cosmetics, pharmacy, medicine, and the food industry.

2.2.3. Food and medicinal uses

In medicine as well as in the cosmetic or food industries, hibiscus calyxes are processed to obtain hibiscus products or to extract molecules of interest such as anthocyanin (Figure 5).

Hibiscus sabdariffa calyxes are used in the food industry mainly to produce hot or fresh drinks, fermented beverages, and wines. Traditional fresh juices are obtained by boiling dried calyxes in drinking water. Insoluble materials are then separated from juice by sieving. Sugar is added as well as mint and some fruits or fruity aroma (pineapple, strawberry, lemon…) to have different flavors, and then the juice is chilled before drinking. Cocktail, syrups, and fruits salad are also made with hibiscus calyx juice. The juice is prepared as seen above and cut fruits are then added to get fruit salad. Hibiscus calyxes or powders are added as natural coloring agents to have red coloration in some cooked foods, pastry foods, puddings and cakes, chocolate (Chumsri et al., 2008; Da-Costa-Rocha et al., 2014; Plotto et al., 2004a; Tsai et al., 2002). The United State of America and the European Union classify anthocyanin as food colorant under the category of fruit (21 CFR 73.250) or vegetable (21 CFR 73.260) and as natural coloring under classification number E163 (Duangmal et al., 2008). In addition, delicious jelly and jam, ice cream of hibiscus calyx are manufactured and appreciated all over the world (Cisse, 2010).

Hibiscus sabdariffa calyx is also known as a medicinal herb due to its composition and special features. Indeed, *Hibiscus sabdariffa* calyxes are used for their diuretic, febrifugal, anthelminthic, antimicrobial, antidiabetic, hypotensive, anti-inflammatory, hepatoprotective and hypocholesterolemic activities and to stimulate the intestinal peristalsis (Da-Costa-Rocha et al., 2014; D'Heureux-Calix and Badrie, 2004; Fullerton et al., 2011; Gómez-Aldapa et al., 2019; Johansen et al., 2005; Lee et al., 2009; Paim et al., 2017; Peng et al., 2011; Pérez-Torres et al., 2013; Ramírez-Rodrigues et al., 2011; Seck et al., 2018). Moreover, anthocyanin molecules can reduce the risks of coronary heart disease due to their antioxidant properties (Cisse, 2010; Johansen et al., 2005; Pérez-Torres et al., 2013; Sinela, 2016).

Figure 5: Composition of *Hibiscus sabdariffa* calyxes, their food and medicinal uses.

2.2.4. Interest in stabilizing *Hibiscus sabdariffa* calyx

The previous parts underlined the fact that the main interest in exploiting hibiscus calyxes lies in their composition and particularly in their richness in anthocyanin, which is responsible for their red color and antioxidant activity. The coloring is the first physical criterion of choice for the customer or consumer, followed by the nutritional quality, more specifically the richness in antioxidants and their health-promoting power (Plotto et al., 2004a). Conscious of the impact of their food on their health, consumers are becoming more demanding, and rightly so. Consumers are also increasingly concerned about the impact of their consumption on the environment (the non-overexploitation of land, the biodiversity) but also about the fair remuneration of farmers and producers. To meet the requirements of this market, but also to avoid food waste, it is necessary to set up stabilization processes that will make it possible to:

- Alleviate the problem of the seasonality of hibiscus;
- Make the product available throughout the year;
- Ensure a long shelf-life for the product;

- Facilitate transport from a producing region to an importing region;
- Make the product accessible;
- Facilitate handling and use of the product;
- Facilitate the extraction of compounds of interest such as anthocyanin.

2.3. Stabilizing processes of *Hibiscus sabdariffa* calyxes

The nutritional value, organoleptic qualities and medicinal benefits attributed to *Hibiscus sabdariffa* calyxes make them an interesting and useful flower. Numerous processing methods have been investigated and set up to develop a stabilized product including drying, extraction, evaporation, and powdering.

2.3.1. Drying

Drying is a way to remove partially or completely water from a material, thus extending its shelf-life. The elimination of water during this process is the result of the simultaneous to heat and mass (water) exchange between the product and its drying environment (Sabarez, 2015). In this way, many fruits, and vegetables like *Hibiscus sabdariffa* calyxes are dried, to reduce water content for better preservation, also to overcome the seasonality issues. Drying is the result of the simultaneous heat and mass exchange between the product and its drying environment (Figure 6). Heat is transferred from the drying environment to the product by different ways depending on the drying type, comprising convection, radiation, conduction, microwave, radio-frequency, Joule (ohmic) heating (Chen and Mujumdar, 2009; Sabarez, 2015). The supplied heat allows increasing the material temperature, inducing a water phase change from water to vapor (due to latent heat at constant temperature) and activating molecular movement. Water is transferred in the opposite direction from the interior to the material surface by capillary flow (liquid) and/or diffusion (liquid or vapor) and diffuses from the surface to the drying medium (vapor).

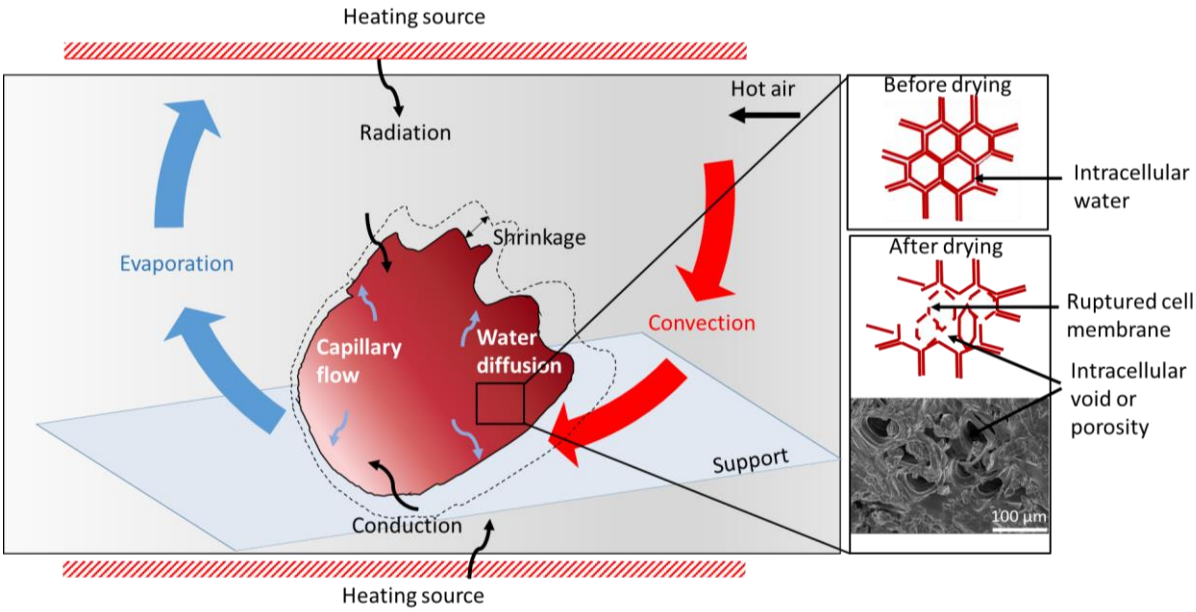


Figure 6: A conceptual representation of the thermal drying process for a hibiscus calyx, adapted from (Sabarez, 2015).

The water vapor exchange between the material surface and the drying medium takes place due to the their positive vapor pressure difference as the driving force (Chen and Mujumdar, 2009; Sabarez, 2015). The water vapor flux (N"$_v$) expressed in kg.m^{-2}.s^{-1} is governed by the equation 1. The drying process continues until an equilibrium between the air relative humidity and the dried product water activity is reached (Sankalpa et al., 2017).

$$N"_v = h_m \times (C_{v,s} - C_{v,\infty}) \qquad (1)$$

Where:

- h$_m$: the mass transfer coefficient, considered as a velocity of mass movement (m.s^{-1}),
- C$_{v,s}$: the water concentration at the moist material surface (kg.m^{-3}),
- C$_{v,\infty}$: the water concentration rate in the surrounding environment (kg.m^{-3}).

The exponentially decreasing curve of the drying rate of hibiscus calyxes can be divided into 2 main stages (Figure 7): a first phase of rapid elimination of water, followed by a second phase marked by a slower water elimination or a quasi-stability of the water content (Tham et al., 2018). This water loss evolution from calyxes is generally similar to that of plant products such as chili, carrot, pumpkin, green pea, garlic (Condorí et al., 2001; Krokida et al., 2003). This typical water loss of plants is inherent to their structure, more precisely to the distribution of water in these products. The hibiscus calyx cells are well-organized, tightly packed, and thick-walled. The plant cells contain water, called intracellular water, which is loosely bound water (80 - 92 g water / 100 g product), while the water circulating between cells, called intercellular water, is free water (6 - 16 g water / 100 g product) (Khan et al., 2016). In addition, the water contained inside the cell wall, which is closely associated with the molecules, is bound water (roughly 6 g water / 100 g product). During the first drying phase, there is a rapid elimination of inter and intracellular water, which are the most available, therefore easily removable. Then comes the stage of drying of bound water that is fixed to the molecules and therefore difficult to evaporate, which leads to a slowing down of the drying.

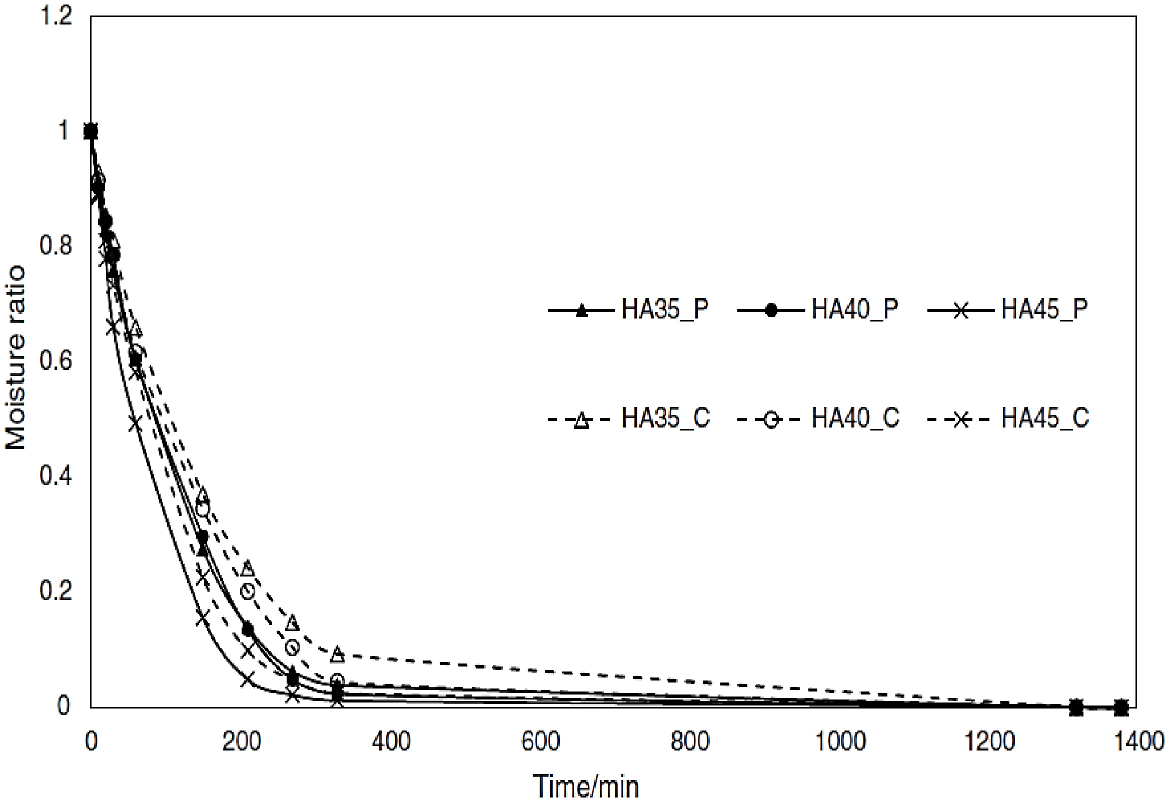


Figure 7: Changes in moisture ratio of *Hibiscus sabdariffa* with time using hot air (HA) strategy at 35, 40, 45 °C, P - Petals, C - Calyx (Tham et al., 2018).

The reduction of water content is also a good way to achieve lightweight and low volume for easy handling and transport. With the technology evolution and the desire for safer products, many types of solar-drying, and other advanced drying technologies (microwave drying, vacuum drying) have been studied. The most common drying treatments of *Hibiscus sabdariffa* in addition to solar-drying are hot air drying and dehumidified-air-drying.

2.3.1.1. Sun-drying

The solar-drying process consists in heating the product by radiation, convection using the sunrays and the air as a heat-carrying fluid to induce the evaporation of material water. In a lesser extent heat is transferred by conduction through the support drying (Figure 8). Therefore this drying process depends on the extrinsic factors like the weather conditions (sun position, duration of sunshine, temperature, humidity, rainy, and air velocity) and intrinsic factors of the product such as the specific surface area, chemical composition, and physical structure (porosity, density, size and shape) (Marnoto, 2014; Tham et al., 2018). *Hibiscus sabdariffa* calyxes, petals, and leaves are usually dried in Africa and Asia by exposure to sunrays. Solar energy is a fundamental energy for these populations since it is accessible, renewable, and free of charge. Fresh calyxes are sun-dried to avoid microbial contamination and have good and long preservation.

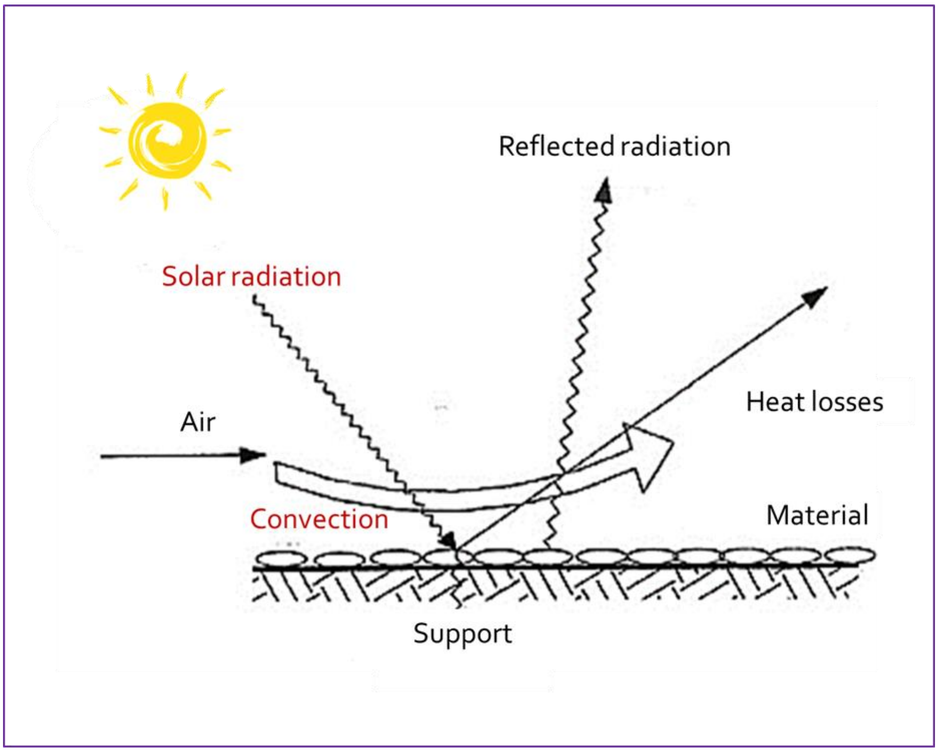


Figure 8: An example of direct sun-drying, adapted from Prakash and Kumar (2013).

Marnoto (2014) classified the solar-drying techniques into 3 groups, direct type, indirect type and intermediate:

- Direct Type: as the name implies, the product is directly exposed to the sun, absorbing heat from sunrays. Products are kept securely in-store during sundown or rainy day. This method needs a long time to dehydrate the material, about 4 to 8, days for having approximately 10 % water content for *Hibiscus sabdariffa* calyxes (Cisse, 2010; Marnoto, 2014), 5 days for *Hibiscus sabdariffa* petals (Marnoto, 2014). However, the exposed products are also exhibited to foreign objects or dust. Moreover, this method cannot be controlled because depending on the weather conditions. The alternation between sun and rain, or sun and cloud may not only lengthen the drying time but also lead to the mushrooming product then rot (Marnoto, 2014; Sankalpa et al., 2017; Tham et al., 2018);
- Indirect Type: unlike the direct type, the product to dry is not exposed to direct sunrays. Drying is done here using air heated and using a solar ray collector, or heat exchanger of a solar dryer. In this way, materials are protected from foreign particles and sun radiations. Some dryers offer the possibility of collecting heat on a clear day, which is then used to ensure overnight drying (Figure 9). Therefore, the drying process becomes faster, the space for drying is reduced by using dryer shelves. *Hibiscus sabdariffa* petals are therefore dried in 2 days (Marnoto, 2014). Tham et al. (2018) studied solar-drying with a transparent thin sheet, which received the sun radiation and transmitted it directly to the entire dryer chamber. The drying rate was unsteady for *Hibiscus sabdariffa* calyxes and petals due to the great variance of temperature 40.16 ± 7.24 °C and the relative humidity (RH) 37.56 ± 15.06 % (Tham et al., 2018). The increase of RH (up to 70 %) observed during the nights, can be the main limit as it leads to microbial contamination;
- Intermediate Type: products are dried by exposure to sunrays and hot air collected in the dryer (Marnoto, 2014), for example, by using a heat pump assistance (Tham et al., 2018). The collected hot air provides the heat in absence of the sun or the case of high air humidity. However, some contaminations could occur, since this method also depends on environmental factors, although in this system the heat is collected and transmitted during sundown to allow a

constant drying. In addition, the drying rate was the highest compare to solar-drying (Tham et al., 2018).

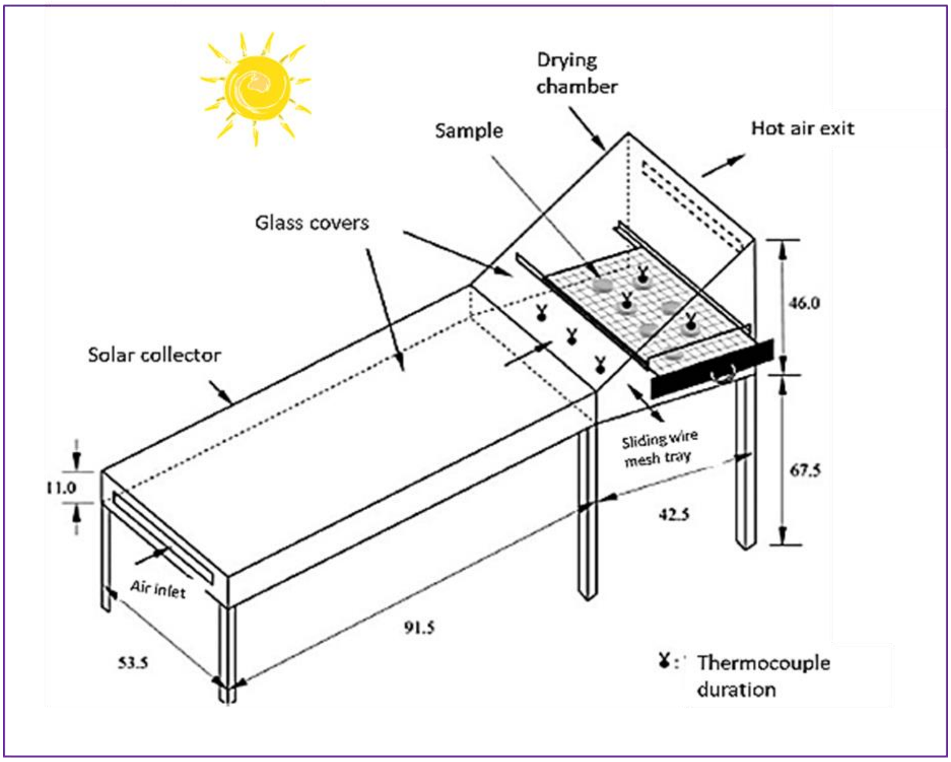


Figure 9: An example of indirect sun-dryer; the collector-dryer assembly consisted of a solar radiation-absorbing surface made of aluminum sheet, painted in matt black, and covered with a glass protection to let the solar radiation through (Tripathy and Kumar, 2009).

2.3.1.2. *Hot air drying*

This process is commonly used in industries because of the facility to handle, the low investment and cost compared to improved technologies (Sankalpa et al., 2017) (Figure 10). Hot air drying is carried out in an oven with convection heating, where warm air is used to remove water from products. Contrary to sun-drying, the water content of the dried products is less variable due to a controlled and confined environment which is independent of weather (Tham et al., 2018). High drying temperatures lead to higher drying rates and water content loss. Kinetic steps differ between the studies may be due to the material physicochemical properties. From the results of oven-dried calyxes of Tham et al. (2018) (35 °C, 40 °C, 45 °C) and Ledesma-Valladolid et al. (2020) (45 °C, 55 °C, 65 °C), 2 main steps are identified: firstly a high drying rate at the beginning of the drying process, followed by a low drying rate or a quasi-constant drying rate unlike the results of Nguyen and Chuyen (2020) (60 °C, 80 °C, 100 °C, 120 °C). Indeed, this difference may be due to the calyx properties such as the structure or the membrane that influences the water transfer during the drying.

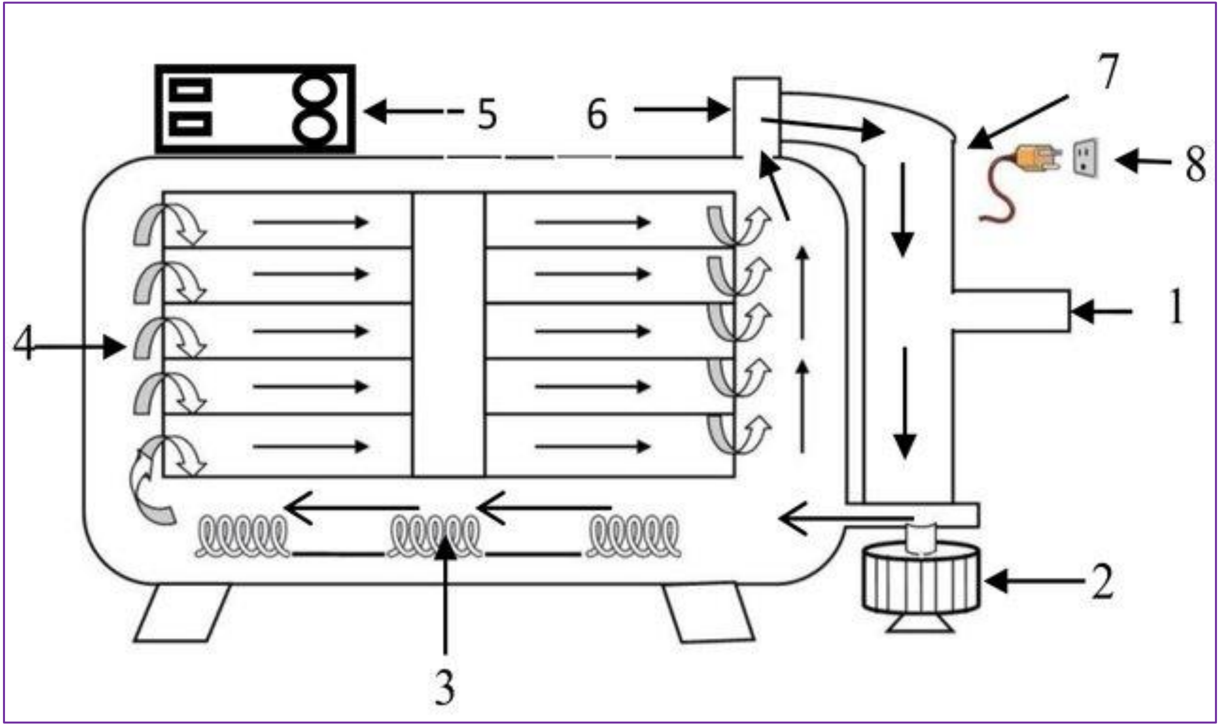


Figure 10: An example of hot air dryer; 1 - inlet air; 2 - blower; 3 - heater; 4 - drying cabinet with removable trays; 5 - control panel; 6 - exhaust air outlet; 7 - air passage line; 8 - power plug (Kumar and Shrivastava, 2017).

2.3.1.3. Dehumidified-air-drying

This process is an advanced technology that consists of drying by keeping the dryer at low temperature and humidity. The principle is to extract the excess humid air exchanged with the moist product, which is placed inside the drying chamber. Humid air is sent to a cold surface of the evaporator, then cooled down to its dew point and condensated. Water is then separated from the dehumidified air which is conveyed in the dryer chamber and the cycle is repeated. Dehumidified-air-drying is not dependent on weather factors and can maintain the optimum drying condition (Tham et al., 2018). This continuous drying process solves the problem of rehydration overnight, an alternative drying solution. Tham et al. (2018) showed that dehumidified-air-drying allows obtaining the highest drying rate of *Hibiscus sabdariffa* flowers compared to oven-drying, solar with intermittent heat pump drying, and solar-drying. Indeed, a greater drying force is created by reducing the air relative humidity to remove moisture on the solid surface. The drying time is then shortened by 45.04 % and 37.60 % in comparison with solar-drying and solar with intermittent heat pump drying respectively. Water activity values obtained by dehumidified-air-drying can be below the maximum threshold, to avoid microbial contamination (aw < 0.6). *Hibiscus sabdariffa* flowers reached a water activity of 0.54 after 20 h of drying at 32.1 ± 2.0 °C (room temperature) and 21.1 ± 3.52 % relative humidity (Tham et al., 2018).

2.3.1.4. Impact of the drying process

Physical modifications may occur during drying, as shrinkage of vegetables like chicory root which became also more porous (Balzarini et al., 2018). Porosity is due to some intercellular voids (spaces) appearing upon the drying treatment. The water removal from the intercellular region leads to the loosening of intercellular bonds and the progressive separation of cells from each other and the removed water is substituted by air (Joardder et al., 2017; Koua et al., 2019). The material porosity is linked to drying conditions and the material characteristics (Figure 11). Highly porous structures are observed when increasing drying temperature, sample size and composition, and decreasing the drying time (Joardder et al., 2016). Fast drying causes a more rapid water removal on the material surface than the interior, so that internal stresses is induced resulting in more cracked and porous material interior (Ramos et al., 2003).

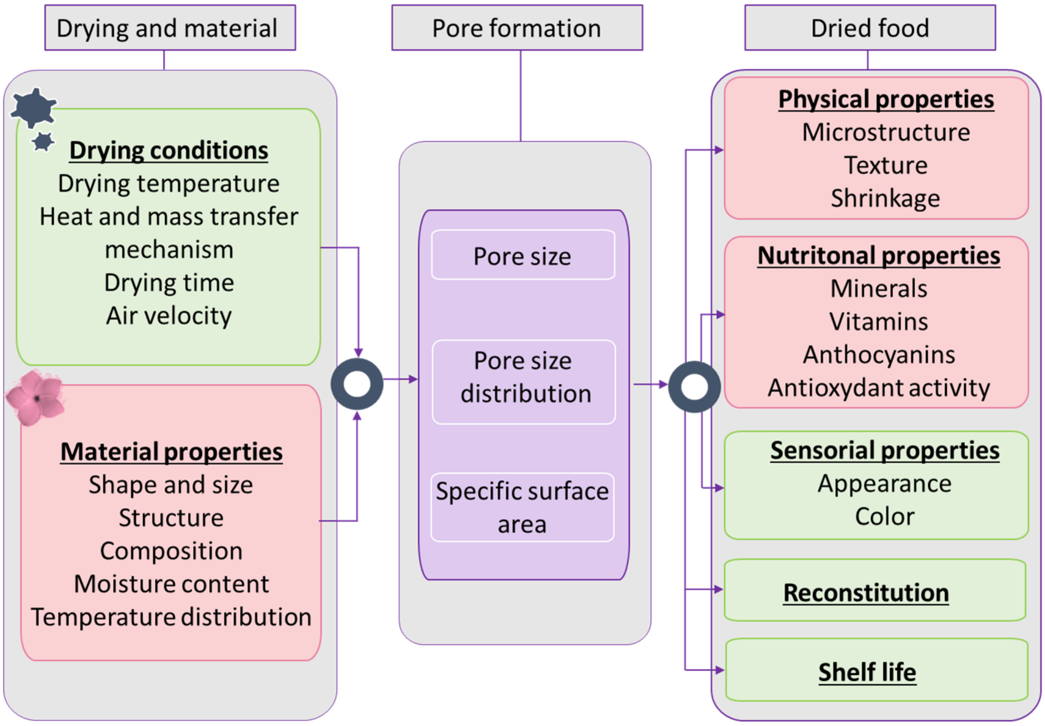


Figure 11: Relationships between drying parameters, material properties, pore formation and dried food qualities, adapted from Joardder et al., 2016.

Shrinkage is determinant in the definition of bulk density. The more the products shrink, the lower the volume, and the higher the bulk density. Like other plants, the epidermis of hibiscus calyxes is formed of cells made up of lamella, wall (primary and secondary), and plasma membrane. The cells of the epidermis of hibiscus calyxes are particularly well-organized, tightly packed, and thick-walled (Figure 12) (Juhari et al., 2021). The cell wall, plasma and the vacuolar membrane are broken down under the effect of drying temperature. Progressively the calyxes fold, become deformed, leading to shrinkage (Juhari et al., 2021). Indeed, the cell liquid exerts a pressure called turgor pressure on the cell membrane keeping the cell in a state of elastic stress, which ensures the stability of the shape, the texture, the firmness, and the crispness of the material. During drying, this turgor pressure is lost and causes the collapse of the plant products (Joardder et al., 2017; Ramos et al., 2003; Seerangurayar et al., 2019). The final structure of the calyxes therefore depends on the type of drying applied. For example, by low temperature freeze-drying, during which the calyxes are frozen then the ice sublimated, the structure was preserved, and the surface was smooth like fresh calyxes. In contrast, the structure of sun-dried calyxes was the most deformed, and shrunk. The temperature, the drying duration may be the reasons (Juhari et al., 2021), but also the material thickness, and the drying rate. A high drying rate, temperature, and air velocity induce more structure deformation (Seeranguravar et al., 2019).

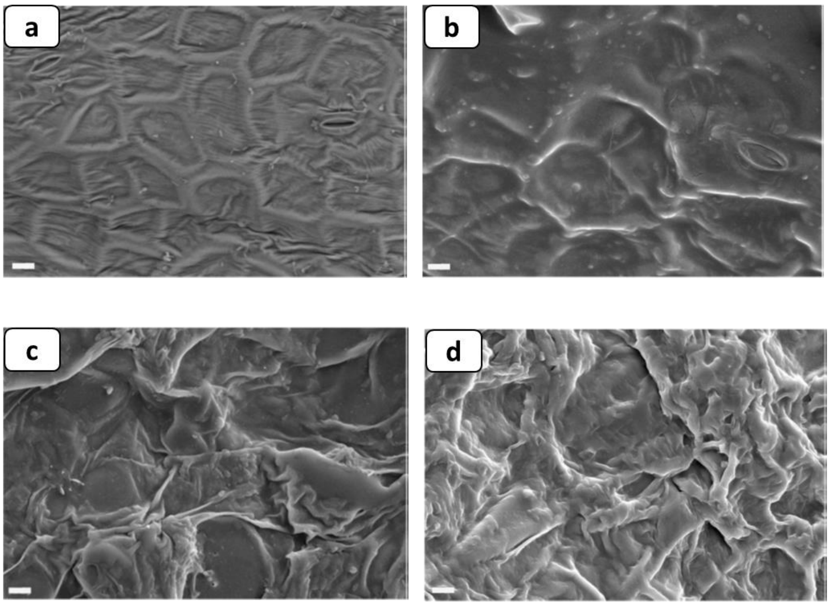


Figure 12: Scanning electron micrographs of the surface of *Hibiscus sabdariffa* calyx samples after different treatments at a magnification of 500 x. Bar = 10 µm; a - fresh; b - freeze-dried; c - oven-dried; d - sun-dried (Juhari et al., 2021).

The color change is one of the prevailing physical effects resulting from hibiscus drying. Dried products can undergo color modification, depending on the drying method applied. Upon drying the colorimetric parameters (lightness (L*), redness (a*), yellowness (b*)) of calyxes may increase. This color change may be justified by the non-enzymatic browning reaction, leading to more reddish and yellowish samples (Tham et al., 2018). Better preservation of the red color (a* = 21.42 ± 2.90) of hibiscus calyxes was obtained by dehumidified drying and the other drying methods studied by Tham et al. (2018) were classified in the followed order: oven-drying > solar with intermittent heat pump drying > solar-drying. In addition, a high level of oxygen can stimulate the browning reaction and increase the saturation (the chroma value) (equation 2) (Jackman et al., 1987; Tham et al., 2018). The oxidized components of the medium can react with anthocyanin, giving rise to colorless or brown products (Jackman et al., 1987). By comparing dried hibiscus calyxes to a fresh one, total color change (equation 3) of 8.39 ± 4.16, 7.28 ± 2.67, 7.27 ± 2.32, and 6.47 ± 2.78 were obtained for oven-drying, solar-drying, dehumidified-air-drying and solar with intermittent heat pump drying respectively.

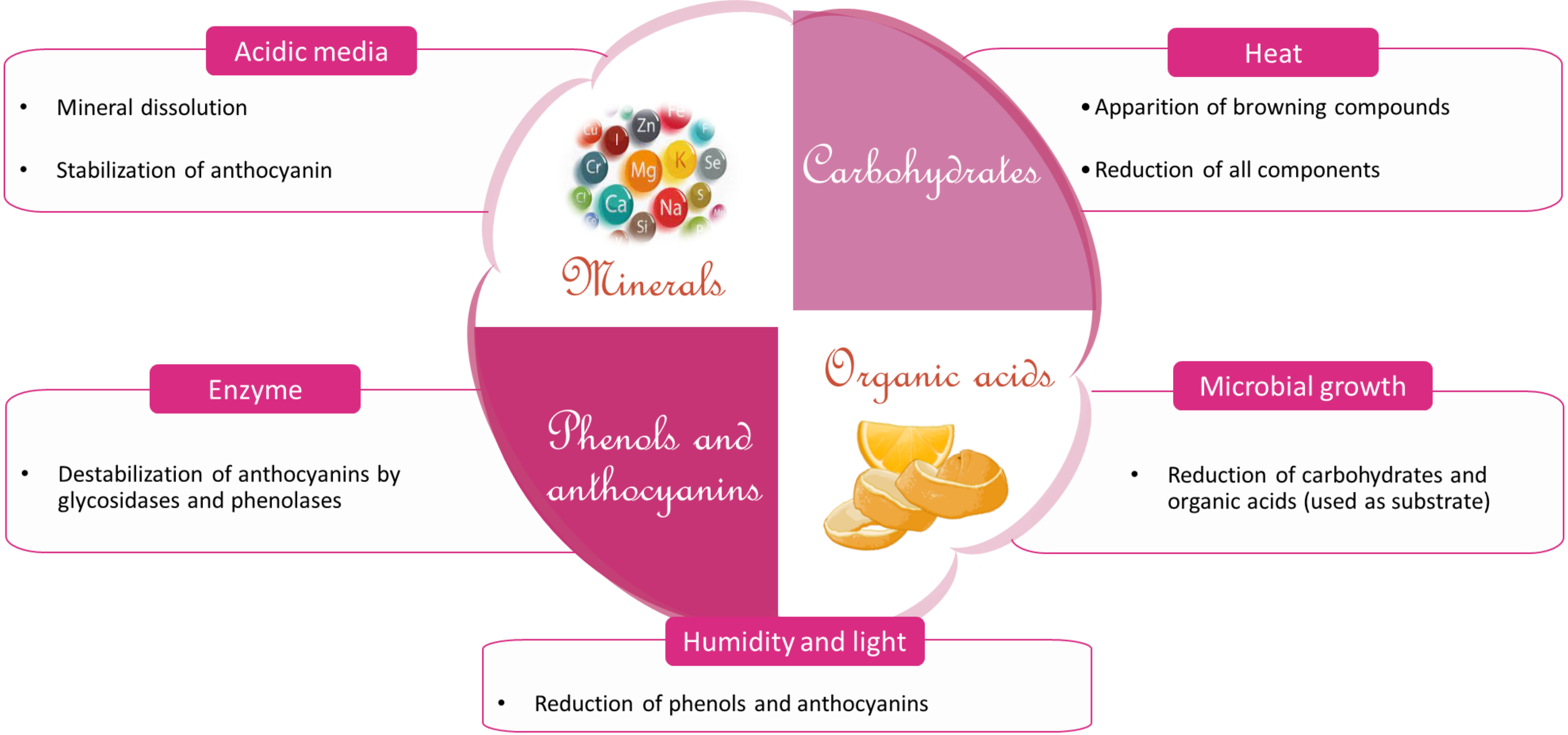
$$C^* = \sqrt{(a^{*2} + b^{*2})} \tag{2}$$

$$\Delta E = \sqrt{(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})} \tag{3}$$

Drying processes may cause degradation of chemical compounds which depends on the drying method, the drying conditions (temperature, air velocity, drying time), and the material (Alibas, 2009; Balzarini et al., 2018; Gong et al., 2007; Sankalpa et al., 2017). Phenolic (protocatechuic, catechin acids) and anthocyanin compounds, responsible for the antioxidant properties of hibiscus calyxes are heat sensitive (Figure 13 and Table 1). Tham et al. (2018) found a greater amount of protocatechuic acid in dehumidified-air-dried calyxes (32 °C) than oven-dried calyxes (30 °C, 35 °C, and 40 °C). Protocatechuic molecules became less stable during heat treatment, leading to their decomposition (Cheng et al., 2014). A loss of catechin content was also observed upon the drying treatment. Catechin molecules were better preserved when drying was carried out at low temperature and humidity (Cheng et al., 2014; Tham et al., 2018). Phenol and anthocyanin molecules of hibiscus calyxes were reduced by 15.3 % and 36.9 % during solar-drying treatment, a non-negligible loss of organic acid was also observed (Sánchez-Feria et al., 2021). Consistent with the investigations of Tham et al. (2018), Balzarini et al. (2018)

showed the reduction of the phenolic compound content of chicory roots with the increase in temperature during vacuum drying. These observations highlight the sensitivity of phenolic compounds to temperature. Dehumidified-air-drying may be the best drying method to result in less degradation of bioactive molecules than solar tunnel drying or oven-drying (Gong et al., 2007; Sankalpa et al., 2017; Tham et al., 2018). Upon solar treatment, fermentation of the organic acids by the natural microflora may partially occurs due to the long-time of drying. Inversely in hot air drying, an inactivation of the microflora could be observed when the temperature is ≥ 50 °C. This temperature coupled to short exposure time (6 to 8 h for hibiscus calyxes), could favor the preservation of organic acids (Sánchez-Feria et al., 2021).

Bioactive components (phenol molecules, anthocyanins) are also subjected to some degradations when increasing the drying temperature. Some investigations showed a decrease in phenol and anthocyanin content when increasing the temperature. When hibiscus calyxes were oven-dried at 60, 80, 100, 120 °C until the water content was below 8 %, Nguyen and Chuyen (2020) observed an important decrease in phenol content. Nevertheless, the phenol content at 80 °C was the highest highlighting the beneficial effect of the short time required at this temperature. Comparing the drying temperature of 80 °C to 60 °C, the latter was coupled with a long drying time leading to longer exposure to high temperature, light, and oxygen, which resulted in greater degradation of phenol molecules. According to the results of Sánchez-Feria et al. (2021) a good preservation of anthocyanin molecule was observed at 50 °C and 70 °C. At 60 °C, a loss of anthocyanins was observed for one variety. When considering the preservation of phenol molecules, the anthocyanins, and the organic acids, they suggested 70 °C as the best temperature. Hibiscus anthocyanins thereby submitted to heat undergo thermal degradation.



Acidic media

- Mineral dissolution
- Stabilization of anthocyanin

Heat

- Apparition of browning compounds
- Reduction of all components

Enzyme

- Destabilization of anthocyanins by glycosidases and phenolases

Microbial growth

- Reduction of carbohydrates and organic acids (used as substrate)

Humidity and light

- Reduction of phenols and anthocyanins

Figure 13: Sensitivity of constituents of *Hibiscus sabdariffa* calyxes.

The consequence is the formation of a phenolic acid arising from the B-ring and an aldehyde resulting from the A-ring (Figure 14) (Sadilova et al., 2006; Seeram et al., 2001; Sinela, 2016).

From an organoleptic point of view, Ramírez-Rodrigues et al. (2011) reported the formation of several volatile compounds due to the drying process, such as hexanal, nonanal, and decanal, products of the oxidation of lipids. For example, dried calyxes presented a high content in 1-octen-3-one responsible for the development of a mushroom and green note, whereas fresh calyxes stand out for their floral and citrus note thanks to linalool (Ramírez-Rodrigues et al., 2011).

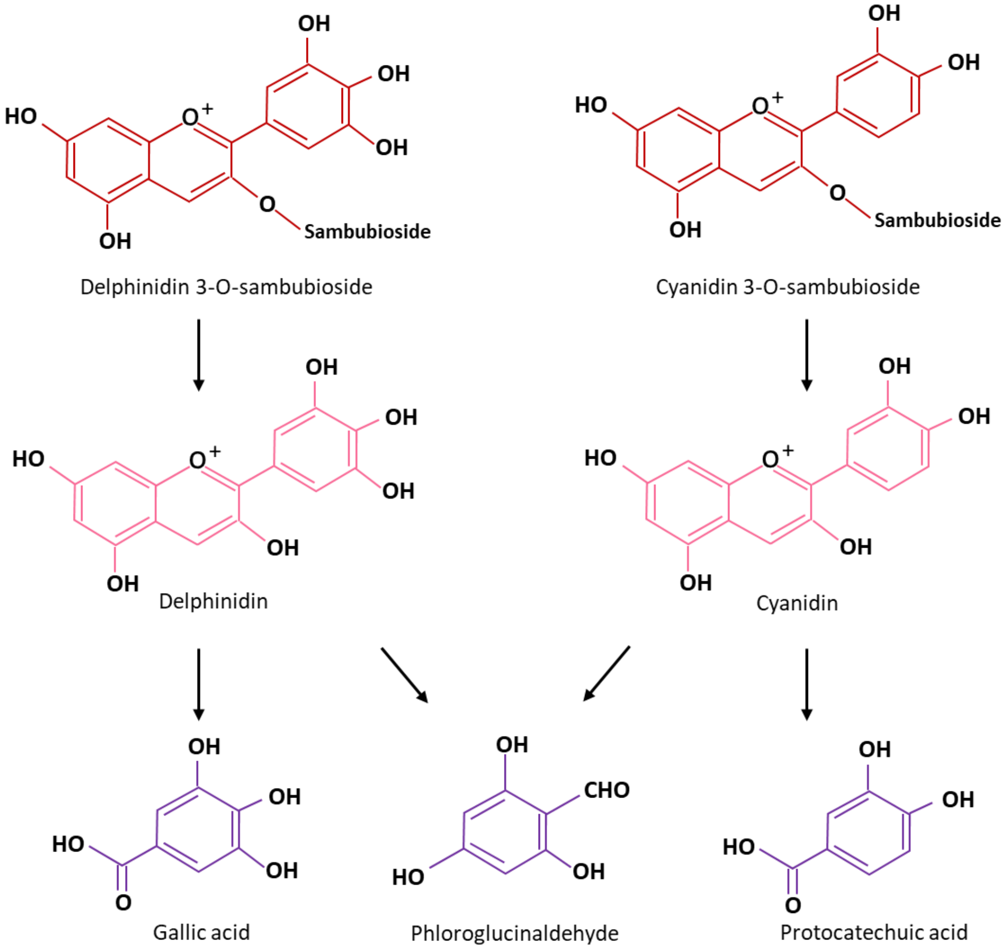


Figure 14: Mechanism of thermal degradation by scission adapted to *Hibiscus sabadariffa* anthocyanins (Sadilova et al., 2006; Seeram et al., 2001; Sinela, 2016).

Table 1: Advantages and disadvantages of the main processing methods of *Hibiscus sabdariffa*.

	Sun-drying	Oven-drying	Spray-drying	Freeze-drying	Grinding	Extraction
Advantages	• Accessible • Free • Renewable	• Easy to handle • Relatively low investment • Controlled conditions	• Cheaper than freezing • Better solubility • Controlled parameters • Controlled particle size • Homogenous particle size • Short time of drying	• Preservation of structure and color • Limited nutrient losses • Good rehydration capacity due to the formation of porous structure in the product	• Simple processing • Increase in the specific surface area • Better bio molecules extraction • Ease of applicability of the product	• Separation of active ingredients or bio molecules of interest
Disadvantages	• High water content (17 – 22 %) • Weather dependent • Biochemical reaction • Possible microbial contaminations • Loss of bio molecules • High labor cost • Large drying area	• Energy and labor intensive drying system • Longer drying time than spray-drying • Browning reaction • Not suitable for thermal sensitive products • Loss of bio molecules	• Stickiness • Caking during drying and storage • Low efficiency due to caking • Loss of bio molecules	• Longer drying time • High energy consumption • high capital cost • More hygroscopic products than spray-dried products	• Maillard reaction • Risks of biomolecules losses • Mix of soluble and insoluble parts of the product	• Risks of thermal degradation • Risks of formation of compounds harmful to the quality of the extract • Instability of bio molecules in the extracts during storage

2.3.2. Solid-liquid extraction

The physical or chemical extraction process consists of the separation of one or many target substances from materials. Solid-liquid extraction is the most used for *Hibiscus sabdariffa* calyxes. Bioactive molecules as anthocyanin are chemically extracted from calyxes to analyze, identify, and use as functional compounds in other products, as well as medicines. This process can also be used to produce beverages, followed by pasteurization or sterilization and storage. Solid-liquid extraction is also applied upstream of some stabilizing processes such as freeze-drying or powdering by spray-drying.

Solvent extraction with water or alcohol (methanol, ethanol) is the most widely used for *Hibiscus sabdariffa* calyxes, including maceration, decoction, infusion (Chumsri et al., 2008; Cisse, 2010; Gonzalez-Palomares et al., 2009; Ramírez-Rodrigues et al., 2011; Sinela, 2016). The extraction process is usually followed by filtration.

Fresh and dried *Hibiscus sabdariffa* calyx infusion was investigated by many authors. Different infusion conditions were employed depending on the goal of the study. Chumsri et al. (2008), Ramírez-Rodrigues et al. (2011) and Cisse (2010) showed that the couple time/temperature, the ratio of the mix, the material composition and size are crucial for the final product characteristics. These parameters impact the physical and chemical properties of the extract including coloration, acidity, anthocyanin and polyphenol content, volatile compounds (Chumsri et al., 2008; Ramírez-Rodrigues et al., 2011). The optimum conditions of fresh *Hibiscus sabdariffa* calyxes extraction based on the best antioxidant activity, preservation of anthocyanins and phenols according to Chumsri et al. (2008) is the ratio 1:2 fresh calyx (g) / water (mL) at 50 °C for 30 min instead of 1:10 dried calyx (g) / water (mL) at 50 °C for 30 min for dried calyxes extract. Dried calyxes need higher water proportion at the same time and temperature than fresh calyxes to be suitably infused, due to the low water content after drying. The lower the ratio, the higher the yield (Cisse, 2010). Anthocyanins extraction from *Hibiscus sabdariffa* or other plants is a function of the solvent, the solvent ratio, temperature, time, stirring. These parameters may modify the chemical composition and properties (pH, coloration) of extracts.

2.3.2.1. Impact of the extraction medium

In a hydrated medium, anthocyanin molecules are in the derivates form of flavylium cation that may change according to the solution pH. The anthocyanin molecules are more stable in a highly acidic medium, at pH < 3 the flavylium cation is predominant, and responsible for the red color (Gradinaru et al., 2003; Sinela, 2016). A pH rise (pH 6.5 to 8) leads to a rapid loss of protons from the flavylium cation shifting the equilibrium towards the quinone form. Up to pH 6, the flavylium cation is hydrated by nucleophilic attack of water leading to equilibrium to the colorless form carbinol. This in turn can generate the chalcone (Figure 15) a colorless molecule (pH > 8) (Bridle and Timberlake, 1997; Chumsri et al., 2008; Cisse, 2010; da Costa et al., 1998; Francis and Markakis, 1989; Markakis, 2012). In addition, the plant matrix affects anthocyanin extraction. In some cases, the anthocyanin molecules can be acylated by other compounds including benzoic acids, resulting in complex formation, thus in the decrease in the hydration capacity of anthocyanin and a color loss (Redus et al., 1999). The color change also depends on the anthocyanin structures as well as the varieties of *Hibiscus sabdariffa* calyxes. Cisse (2010) reported that the anthocyanin from the Vimto varieties was less heat sensitive than Koor and Thaï varieties. In comparison with other fruit juices, Vimto varieties extracts may be more vulnerable than blackberry juice but less sensitive than blood orange. Yan et al. (2020) studying grapes reported that the biosynthesis could favor some stable forms of anthocyanin such as methoxylated anthocyanins and flavonols, as well as acylated anthocyanins, depending on the varieties. The chemical and thermal stability of anthocyanins is enhanced by these methoxylation and acylation phenomena (Liu et al., 2018; Yan et al., 2020) and could be the reason for the differences in temperature sensitivity of hibiscus varieties anthocyanin during treatment. Color reduction may occur in presence of pigment-copigment complex because the flavylium ion become more sensitive to hydration, leading to colorless molecules (carbinol and chalcone) (Figure 15) (Brouillard and Dangles, 1994).

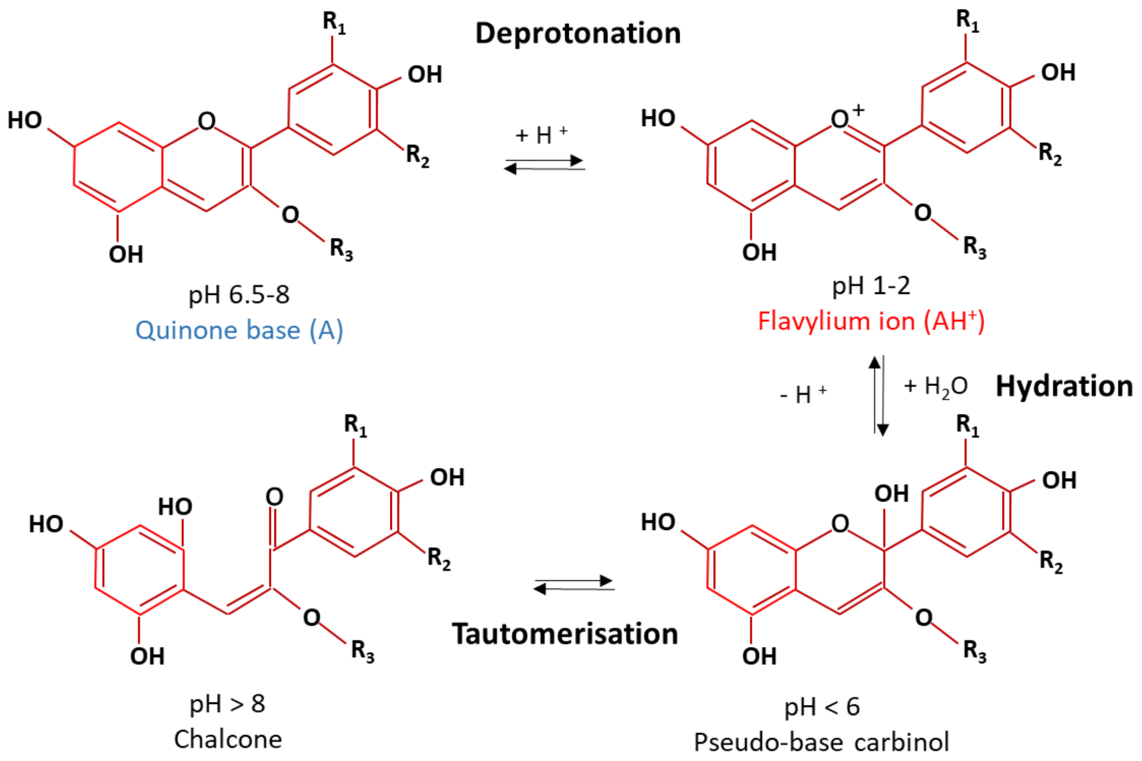


Figure 15: Main forms of anthocyanin in equilibrium in aqueous medium (Brouillard et al., 1989).

Moreover, the ratio increment (calyxes (g) / water (mL)) of fresh hibiscus calyxes favored an increase in the red color intensity, acidity, total soluble solids, anthocyanins, total phenolic contents and antioxidant activity of extracts (Chumsri et al., 2008). Similar observations were reported for dried calyxes with the increase of water content (low ratio) but the acidity and the Brix were reduced, in contrast to the other compounds (soluble material, anthocyanin and phenolic compounds) (Chumsri et al., 2008). In line with these authors, Nguyen and Chuyen (2020) showed that the rise of the water portion (from 8 mL to 10 mL for 1 g dried calyxes) improved the extraction of phenols but more water portion decrease the phenol content. Indeed, more solvent cannot increase proportionally the diffusion rate because the amount of solute is limited. Following the best extraction conditions (i.e. 1:2 and 1:10 calyxes (g) / water (mL) at 50 °C for 30 min for fresh and dried calyxes respectively), Chumsri et al. (2008) observed some great differences in total anthocyanin 45.13 and 502.33 mg / 100 g for fresh and dried hibiscus extracts. Similarly, the dried calyx extract is the richest in phenol content 43 mg / g compared to 22.25 mg / g gallic acid for fresh calyx extract. This may be due to the lower water content of dried calyxes than fresh calyxes, allowing a concentration of compounds. In addition, Chumsri et al. (2008) noted a best antioxidant activity for dried calyx extracts for an identical ratio (1:10) than fresh calyx extracts. The antioxidant activity seemed to increase with the acidity.

2.3.2.2. Impact of temperature and time

The couple temperature/time has an important role in the extraction process. High extraction temperature or long time induce high soluble extraction but also cause instability of food (Chumsri et al., 2008; Cisse, 2010; Eroğlu et al., 2018). On the one hand, high temperature coupled with the right time and particle size of material favored extraction by increasing the diffusion coefficient of anthocyanin (Cisse, 2010). However, high-temperature extraction may lead to an alteration of phenolic and anthocyanin compounds, ascorbic acids of hibiscus calyxes (Table 1) and reduce the antioxidant power (Chumsri et al., 2008; Eroğlu et al., 2018). Special attention should be paid to the optimal temperature because this choice depends on molecule target to preserve, and the temperature range considered in each study. For example, Nguyen and Chuyen (2020) recommended 90 °C instead of 80 °C and 100 °C, while Chumsri et al. (2008) found 50 °C as optimal temperature. These latter authors focused on phenolic and anthocyanins compounds, while Nguyen and Chuyen (2020) worked on the soluble phenolic compounds. The increment of extraction time has similar impacts on fresh and dried calyx extracts, it may result in a reduction of total anthocyanin contents and antioxidant activity (Chumsri et al., 2008; Eroğlu et al., 2018). These reductions were accompanied by a decline of the color parameter of extracts like the lightness (Chumsri et al., 2008). Besides anthocyanin molecules degradation, there may be a simultaneous development of non-enzymatic browning product modifying the coloration (Cisse, 2010). However, some authors noted better stability over time of anthocyanin at low water activity, hence the idea of stabilizing the product by reducing its water activity (Sinela, 2016). On the other hand, the use of hot water for hibiscus calyx infusions favored the production of more aldehydic volatiles whereas fresh extracts were rich in alcoholic volatile compounds (Ramírez-Rodrigues et al., 2011).

2.3.3. Evaporation

Evaporation is used to concentrate, and to reduce the moisture and the water activity allowing better preservation of the product. Liquids are also evaporated to facilitate and achieve effective drying of hibiscus extract. In this last case, evaporation is considered as a pre-treatment (Eroğlu et al., 2018; Gonzalez-Palomares et al., 2009). Chumsri et al. (2008) conducted the concentration of hibiscus extracts to 25 °Brix by vacuum (58 661.84 Pa at 70 °C) and atmospheric evaporation to get concentrated juice. Greater phytochemicals were obtained with vacuum evaporation including anthocyanin molecules in addition to better antioxidant activity.

2.3.4. Powder production

2.3.4.1. Droplet conversion

2.3.4.1.1 Spray-drying

Spray-drying is the transformation of a concentrated liquid into a dried particulate form by spraying the feed into a hot drying gas (Figure 16). Spray-drying involves entrainment. When a wet product is placed in a sufficiently hot and dry gas, a temperature and partial water pressure gradient spontaneously occurs leading to a heat transfer (from the air to the product), a reverse water transfer occurs due to the difference in partial water pressure between the air and the surface of the product. The gas serves as both a heat transfer fluid and a carrier gas for the elimination of water vapor (Schuck et al., 2012a). When fed into the drying chamber, the solution is firstly atomized, otherwise, it is disintegrated into tiny droplets. The spray-air contact is the second step, which consists on send hot air in the chamber either by co-current or counter-current flow (Anandharamakrishnan and Ishwarya, 2015). Spray-drying is the most applied process to obtain powders and encapsulate pigments as anthocyanin (Djaeni et al., 2018; Gonzalez-Palomares et al., 2009; Idham et al., 2012). Indeed, it is a good preservation method of biomolecules (Beristain and Vernon-Carter, 1995; Ré, 1998). In addition, *Hibiscus sabdariffa* extracts are spray-dried to have instant powders (Gonzalez-Palomares et al., 2009; Nguyen, 2018). To achieve this processing, it is worth noting that spray-drying necessarily comes after a hibiscus extraction step and ideally, after a concentration step as well, because it reduces the energy consumption therefore the energy cost of spray-drying (Moejes et al., 2018). According to the good acceptability sensory (based on the greatest volatile compounds), Gonzalez-Palomares et al. (2009) in agreement with Al-Kahtani and Hassan (1990), classified as the best, powders obtained at an inlet temperature ranging from 190 to 200 °C and outlet temperature of 80 °C. However, powder with the smallest ascorbic acid and total monomeric anthocyanin losses (26.67 and 29.58 %, respectively), and the highest product yield 81.96 % (the amount of instant powder obtained from 1 kg of raw material), were obtained at 130 °C and 85 °C inlet and outlet air temperature respectively, in the study of Eroğlu et al. (2018). The optimal conditions depend on the material chemical composition and the aim of the studies or the molecules to preserve.

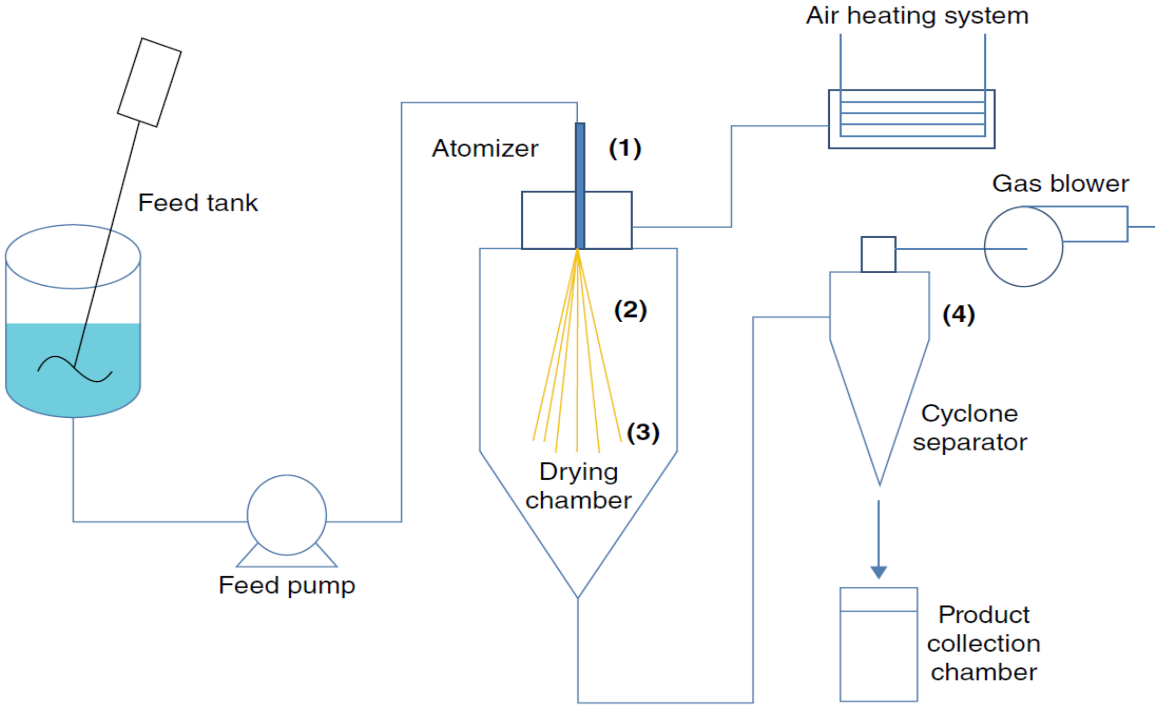


Figure 16: Principle of spray-drying process for powder production; 1 - Atomization, 2 - Contact with hot air, 3 - water evaporation (Anandharamakrishnan and Ishwarya, 2015).

Physical impacts. Spray-drying requires high inlet temperatures to remove water from materials in a short time. Even if the product surface temperature remains lower than the drying air inlet temperature (wet bulb temperature of air as long as a_w = 1), this temperature may be higher than the glass transition temperature impairing the powders, particularly those composed of amorphous particles and sugar-rich materials. Temperature rise may lead to stickiness, which results in caking, and powder adhesion to the dryer surface (Table 1). Indeed, the increase in temperature could induce the passage of amorphous particles into sticky particles (glassy transition) when the glass transition temperature (Tg) is reached and/or exceeded (Eroğlu et al., 2018; Largo Avila et al., 2015). For spray-dried hibiscus, Langrish and Chiou (2008) found a Tg ranging from 50 °C (water content = 10.74 g / 100 g) to 73 °C (water content = 8.30 g / 100 g). In addition, some materials may reach their melting point causing adhesion to the dryer surface (Langrish and Chiou, 2008). This phenomenon is common to products rich in carbohydrates or lipids, leads to a loss of powder, and results in lower yield (Eroğlu et al., 2018; Largo Avila et al., 2015).

Chemical impacts. The pH of hibiscus powders did not differ significantly from that of extracts regardless of the heat treatment (Gonzalez-Palomares et al., 2009), but some components such as anthocyanin molecules, ascorbic acid were not as stable and could be altered. Eroğlu et al. (2018) assessed a loss of 36.9 % and 49.2 % anthocyanin and ascorbic acid compared to raw materials, in instant hibiscus blended rosehip powder. In addition, some reactions may occur and result in the formation of new products during spray-drying (Eroğlu et al., 2018). Analyzing *Hibiscus sabdariffa* calyx powders, Gonzalez-Palomares et al. (2009) and Ramírez-Rodrigues et al. (2011) found furfural molecule a product of non-enzymatic reaction, furanic linalool oxide, cis-linalool oxide, eugenol molecules. The formation of these molecules and the loss of initial molecules means that some degradations of initial products and Maillard reaction occur during the process due to the temperature and the presence of oxygen (Cassol and Noreña, 2021; Gonzalez-Palomares et al., 2009). Increasing the temperature led to more production of these compounds and the loss or degradation of the initial molecules including anthocyanin, acid ascorbic resulting in a poor antioxidant activity (Eroğlu et al., 2018; Nur Fitriani et al., 2021). Carrier agents such as maltodextrin, gum Arabic, pectin, carboxymethyl cellulose, whey protein may be added to the extracts before spray-drying to encapsulate biomolecules and avoid their loss (Barbosa et al., 2015; Cassol and Noreña, 2021; Díaz-Bandera et al., 2015; Eroğlu et al., 2018). However, the concentration of the carrier agent could affect the biodisponibility of compounds of interest. For example, Eroğlu et al. (2018) highlighted for hibiscus blended rosehip powder a reduction in ascorbic acid contents with the increase in maltodextrin although the product yield increased.

2.3.4.1.2 Freeze-drying

Freeze-drying is another drying method where the material is frozen and then water is removed by sublimation (Figure 17). It differs from the spray-drying process that removes the water content from the product by entrainment. Small parts of the material are rapidly frozen to produce ice crystals, then the surrounding pressure is lowered (below 610 Pa) and the ice sublimates to water vapor when heat is slowly applied to the frozen material (Fellows, 2017). In liquid foods that do not have a cellular structure, slow freezing is used to form a network of large ice crystals. Channels formed by the sublimed ice allow the vapor to escape more quickly than in solid foods (Fellows, 2017). Freeze-drying requires low temperatures, it is successful for most foods and recommended for heat-sensitive products (Fellows, 2017; Kuck and Noreña, 2016). However, it is an onerous drying method up to five times more expensive than conventional drying (Table 1), due to the necessity of previously freezing the sample, followed by ice sublimation at low pressures (Cassol and Noreña, 2021; Fellows, 2017). *Hibiscus sabdariffa* calyxes are firstly transformed into extracts before being freeze-dried (Cassol and Noreña, 2021; Duangmal et al., 2008; Gradinaru et al., 2003; Zheng et al., 2021) and can be ground as post-treatment to get powders. Freeze-drying is applied to better preserve the bioactive molecules of hibiscus calyxes. To improve the stability of these compounds, the effects of some stabilizers or carriers (maltodextrin, trehalose, pullulan, whey protein isolate) on the stability of the biomolecules have been investigated (Cassol and Noreña, 2021; Duangmal et al., 2008; Gradinaru et al., 2003).

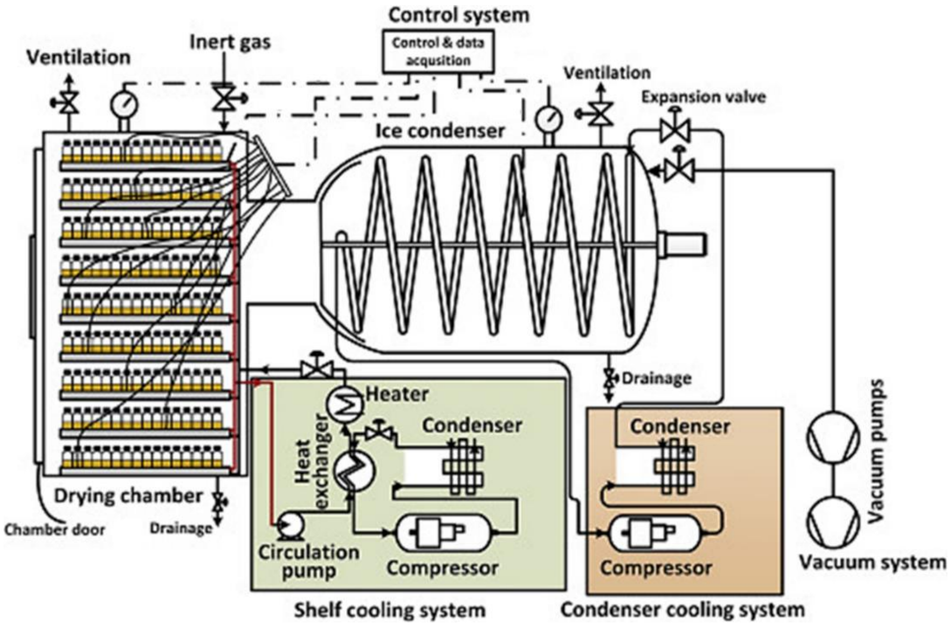


Figure 17: Scheme of freeze-drying principle (Assegehegn et al., 2020).

Physical impacts. The freeze-dried hibiscus extracts studied by Duangmal et al. (2008) became flakes, with good preservation of the color. They observed a total color change (ΔE) of 1.9 ± 0.1 units and explained this modification by the degradation of anthocyanin (about 5 %) which was correlated to lightness and chroma. This color change is delayed when using carrier agents such as maltodextrin (Duangmal et al., 2008). The freeze-drying-induced hibiscus powder because of a better-preserved and appealing red color could attract more consumers (Table 1). The chroma change was more pronounced than the other color parameters (light and hue) and continued to decrease during 105 days storage at 30 °C resulting in duller samples (Duangmal et al., 2008). Chroma value has been proved to be a good indicator of anthocyanins content due to the strong correlation to this molecules content (Duangmal et al., 2008; Mozetič et al., 2004). Since freeze-dried products are in solid form, the dried products may be ground to enable easy use.

Chemical impacts. The great capacity of freeze-drying to preserve the product quality such as biomolecules, smell, and the flavor was reported by several authors including Fellows (2017), Duangmal et al. (2008) and Gong et al. (2007). Freeze-drying was applied to produce cabbage powder, showing a higher vitamin C, about 1.7 times greater than hot air drying processing (Gong et al., 2007). In addition, Duangmal et al. (2008) studying freeze-drying of hibiscus extract (water – 95 % ethanol as solvent) under 0.05 hPa vacuum for 15 h, assessed a high anthocyanin retention with 95.08 ± 5.18 %. Water activity plays a non-neglected role in the stability of anthocyanin (Brønnum-Hansen et al., 1985; Duangmal et al., 2008; Thakur and Arya, 1989). Duangmal et al. (2008) obtained the highest hibiscus anthocyanin stability at the lowest water activity (aw = 0.11) for the freeze-dried extract with maltodextrin (3 g / 100 mL). This is explained by the reduction of the reactant mobility due to the addition of maltodextrin which might impact the hygroscopicity of products (Duangmal et al., 2008). In agreement with this study, Thakur and Arya (1989) reported a loss of blue grape anthocyanins with high water activity.

Furthermore, after freeze-drying, special attention must be paid to the quality of the product during storage because the anthocyanin molecules could be altered with time during storage. To overcome this issue some works investigated the addition of stabilizers in extracts and studied their effect on the freeze-dried product during storage. In the case of *Hibiscus* *sabdariffa* for example, the effect of some stabilizers including maltodextrin, trehalose, pullulan, whey protein isolate on the anthocyanin stability was studied (Cassol and Noreña, 2021; Duangmal et al., 2008; Gradinaru et al., 2003). The addition of maltodextrin and trehalose favored better stability of hibiscus anthocyanin during storage at 30 °C for 105 days, and the half-time (the time needed for 50 % degradation of anthocyanins) was evaluated (Duangmal et al., 2008). Duangmal et al. (2008) recorded a half-time ($t_{1/2}$) of 92, 97, 247 days for freeze-dried hibiscus extract, freeze-dried hibiscus extract with trehalose (3 g / 100 mL), and freeze-dried hibiscus extract with maltodextrin (3 g / 100 mL). The increase (from 2 to 3 g / mL) in trehalose concentration induced higher loss in anthocyanin content in contrast to maltodextrin which provided better stability of anthocyanin during storage (Duangmal et al., 2008). Indeed, in any medium (liquid or not), maltodextrins bind to molecules in contrast to copigmentation which occurs in aqueous conditions (Brouillard et al., 1989). Dextrins react with the flavylium cation derivatives in the extract giving complexation products which prevent the anthocyanin transformation in other less stable forms (Chandra et al., 1993).

2.3.4.2. Size reduction

2.3.4.2.1 Dry grinding

The dry grinding process consists of splitting a product into small pieces after applying mechanical stress using a special device. During grinding, material overcomes deformations leading to breakage. The first deformation may be elastic, in which case the material can be restored to its original shape (Chamayou and Fages, 2016). With the rise of the applied stress, the material may reach its yield strength. Exceed this limit, the deformation is termed plastic deformation meaning that the material cannot regain its initial shape. The plastic deformation continues with additional applied stress and reaches the breaking point, therefore the material breaks (Chamayou and Fages, 2016). Several authors Chamayou and Fages (2016) and Hulin (1990) reported that a broken material using low energy and without the elastic deformation step is called brittle material. Chamayou and Fages (2016) named malleable materials, products broken after high deformations, mainly plastic deformations. The intermediate class between brittle and malleable is semi-brittle materials.

Moreover, the grinding mode influences the fracture of materials and varies with the grinder. The different fragmentation modes are compression, cutting, shearing, attrition, and impact crushing (Figure 18).

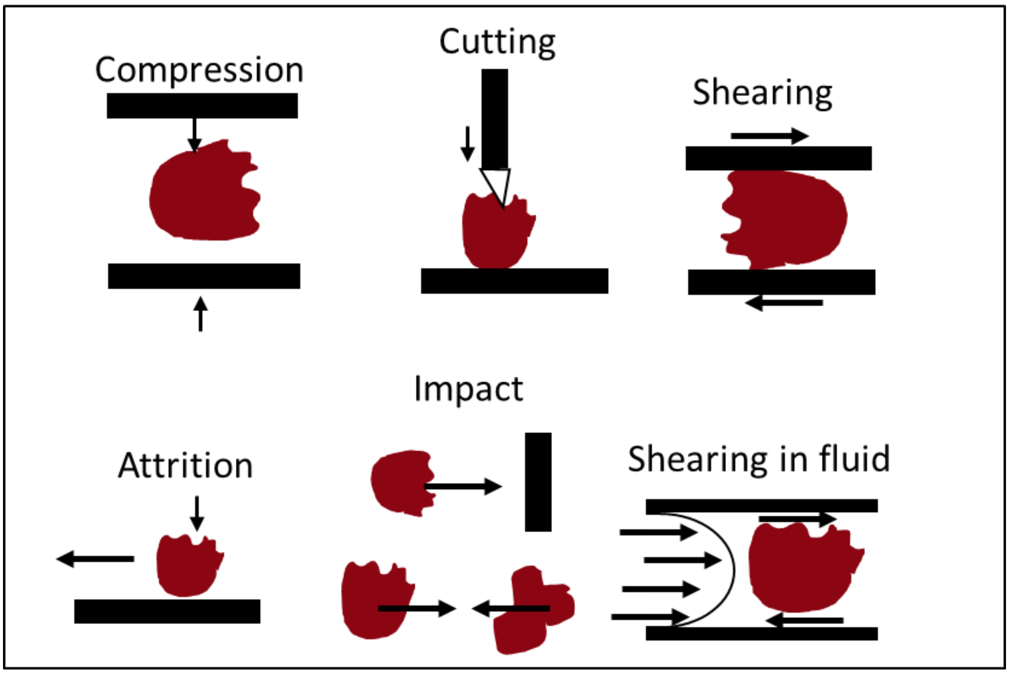


Figure 18: Solid fragmentation modes, adapted from Chamayou and Fages (2016).

Grinding by compression consists to apply pressure to the product with a rotor to reach the breaking point (Esnault, 2015). In the food industry, a roller mill has been developed for this size reduction mechanism and it is used for example for wheat, chocolate refining (Balasubramanian et al., 2012; Esnault, 2015; Karam et al., 2016). Cutting and shearing are grinding methods allowing to reduce products in small particle size and can be applied to elastic materials. Knives and shredders mills are used for cutting and shearing, and colloids or disc mills are used for attrition grinding. The latter method induces fragmentation by the friction of tough particles. Grinding by impact allows achieving different particle sizes, ranging from the largest particles (employing hammer mills) to the ultrafine particles (with jet or fine or pin mills).

Three mechanisms of fragmentation are distinguished depending on the geometry of the grinder, the mode of fragmentation and the energy used to break the materials (Chamayou and Fages, 2016; Hulin, 1990):

- Abrasion: it consists of an erosion of the surface of the particles. The energy is so low that only a few fragments are removed. Two large populations are generated, the first of a size close to that of the abraded particle, the second made up of much finer than the initial population;

- Destructive breaking: it results in energy input, sufficiently intense to generate stress that far exceeds the breaking point. Particles thus generated are small compared to the parent particle and the size distribution of these fragments is very spread out;
- Cleavage: it is an intermediate phenomenon between abrasion and destructive breaking. It causes the production of particles of the same order of magnitude as the parent particle; it results from energy just sufficient to propagate pre-existing fractures in the treated material.

The particle size is therefore dependent on intrinsic parameters of products as physicochemical composition and the structure of materials. It depends also on the extrinsic parameters including the device, the system used to break and the conditions of grinding like temperature, relative humidity and grinding speed.

Materials with high water content, fibers and/or sugars content as *Hibiscus sabdariffa* calyxes are difficult to grind unlike products rich in minerals, fat, proteins and therefore need high energy to be ground (Baggenstoss et al., 2008; Becker et al., 2016; Zaiter et al., 2016). This high presence of fiber could result in a solubility reduction of the hibiscus powders. Dry grinding should be preceded by drying for products with high water content to enhance the grinding process and obtain finer powder. During the grinding process, the energy used to grind may be converted to thermal energy, increasing the temperature of the mill, which in turn heats the ground product. This temperature rise may cause degradation of the powder compound and modify the functional properties according to the material thermal sensitivity. In that sense, other grinding methods have been developed to maintain the temperature or to avoid heating during the fragmentation. Some cold grinding methods implemented, allow to maintain the process at the optimum temperatures, including cryogenic grinding, grinding with water cooling, grinding with liquid nitrogen cooling (Ghodki and Goswami, 2016; Karam et al., 2016; Sankalpa et al., 2017; Singh et al., 2018). In addition, the risk of browning may be reduced by applying vacuum grinding (Kim et al., 2017).

It can be seen that some authors mainly applied the ambient grinding of *Hibiscus sabdariffa* calyxes as a prerequisite, powders are then used to facilitate further processes such as anthocyanin or phenol extraction, rehydration or encapsulation (Builders et al., 2010; Cid-

Ortega and Guerrero-Beltran, 2014; Deli et al., 2019b; Tan and Sulaiman, 2020). Indeed, a reduction in the size of calyxes from 2 cm to 150 µm resulted in a considerable reduction in extraction time from several hours to less than 10 min with an increase in yields (Cisse, 2010).

Physical impacts. The increment of temperature during the grinding may impair the powder color by leading to changes in redness, yellowness and lightness (coloration parameters) (Ghodki and Goswami, 2016; Singh et al., 2018). Grinding-induced powders at room temperature can be heterogeneous, with different populations unlike the homogeneous powders obtained by grinding at low temperature (Deli et al., 2019b; Ghodki and Goswami, 2016; Sankalpa et al., 2017; Singh et al., 2018). A homogeneous population of large particles is more conducive to good flowability than a heterogeneous population because the fine particles fit into the interparticle porosities preventing their flowability (Petit et al., 2017). Singh et al. (2018) studying ball milling of king chilli at ambient temperatures 30 ± 2 °C and low temperature - 90 ± 3 °C, observed 27.56 % finer powder at low temperature compared to ambient grinding. Indeed, at cryogenic temperature particles were more breakable (below the glass transition temperature) allowing to have smaller milling-induced particles (Singh et al., 2018). This rise of particle fineness when dropping the temperature is also highlighted by Ghodki and Goswami (2016) having worked on hammer milling of black pepper. Fine particles may be more regular, spherical and smoother (Deli et al., 2019b; Singh et al., 2018). However, this observation is not general, as high roughness and low sphericity can be observed by lowering the temperature (Ghodki and Goswami, 2016). The fine, rough, irregular shaped particle can lead to a decrease in inter particular distance and an increase in the number of inter particular contact points, which enhances powder cohesion and impairs the powder flowability (Fitzpatrick et al., 2007; Ghodki and Goswami, 2016; Gnagne et al., 2017; Petit et al., 2017; Singh et al., 2018).

Chemical impacts. The decrease in temperature applied to avoid heating during grinding is not without effect, this may lead to an increase in the powder water content. The ambient air steam met the powder cold surface and led to water condensation increasing water content and water activity of powders (Ghodki and Goswami, 2016). Water activity increased also when the particle size was reduced as observed Cid-Ortega and Guerrero-Beltran (2014) and Deli et al. (2019) on B. senegalensis and *Hibiscus sabdariffa* powders obtained by grinding and sieving. Indeed, the reduction in small size leads to an increase in the surface area of particles (Deli et al., 2019b; Ghodki and Goswami, 2016; Singh et al., 2018) leading to more exchange with the surrounding air and increased interactions with air humidity, enhancing water absorption, therefore increasing water activity (Petit et al., 2017).

As well, nutrients, bioactive molecules are more available in fine particles but may be exposed to the environment (Table 1) (Deli et al., 2019b; Ghodki and Goswami, 2016; Singh et al., 2018). The chemical properties of ground vegetables including hibiscus calyxes vary according to the particle size (Deli et al., 2019b). Minerals, proteins, and lipids content in *Hibiscus sabdariffa* calyx sieved powders were more important in small particle powders than in large particle powders. This observation is in agreement with studies on Hypericum perforatum and Achillea millefolium powders, where small particle powders were the richest in minerals (Becker et al., 2016). However, the inverse results can be observed for phytochemicals compounds. Deli et al. (2019) reported also that the smaller the particles, the higher the loss in phytochemicals. This can be explained by the great specific surface area of fine particles, which enhance more exchange with the surrounded environment, leading to phytochemicals loss. Nevertheless, this trend depends on the biological material structure and the sensitivity of the bioactive molecules, since in the same study Deli et al. (2019) found higher phenols and flavonoid contents in the finest powder of Boscia senegalesis, unlike the finest *Hibiscus sabdariffa* powder which contained the lowest quantity of flavonoid and the greatest phenol content. Moreover, these differences may be explained by the sensitivity of phytochemicals to temperature during the grinding process. Some studies highlight the phytochemicals degradation due to the heat during the grinding process (Hu et al., 2012).

A summary graph is proposed to overview the influences of processing methods on the quality of hibiscus products (Figure 19).

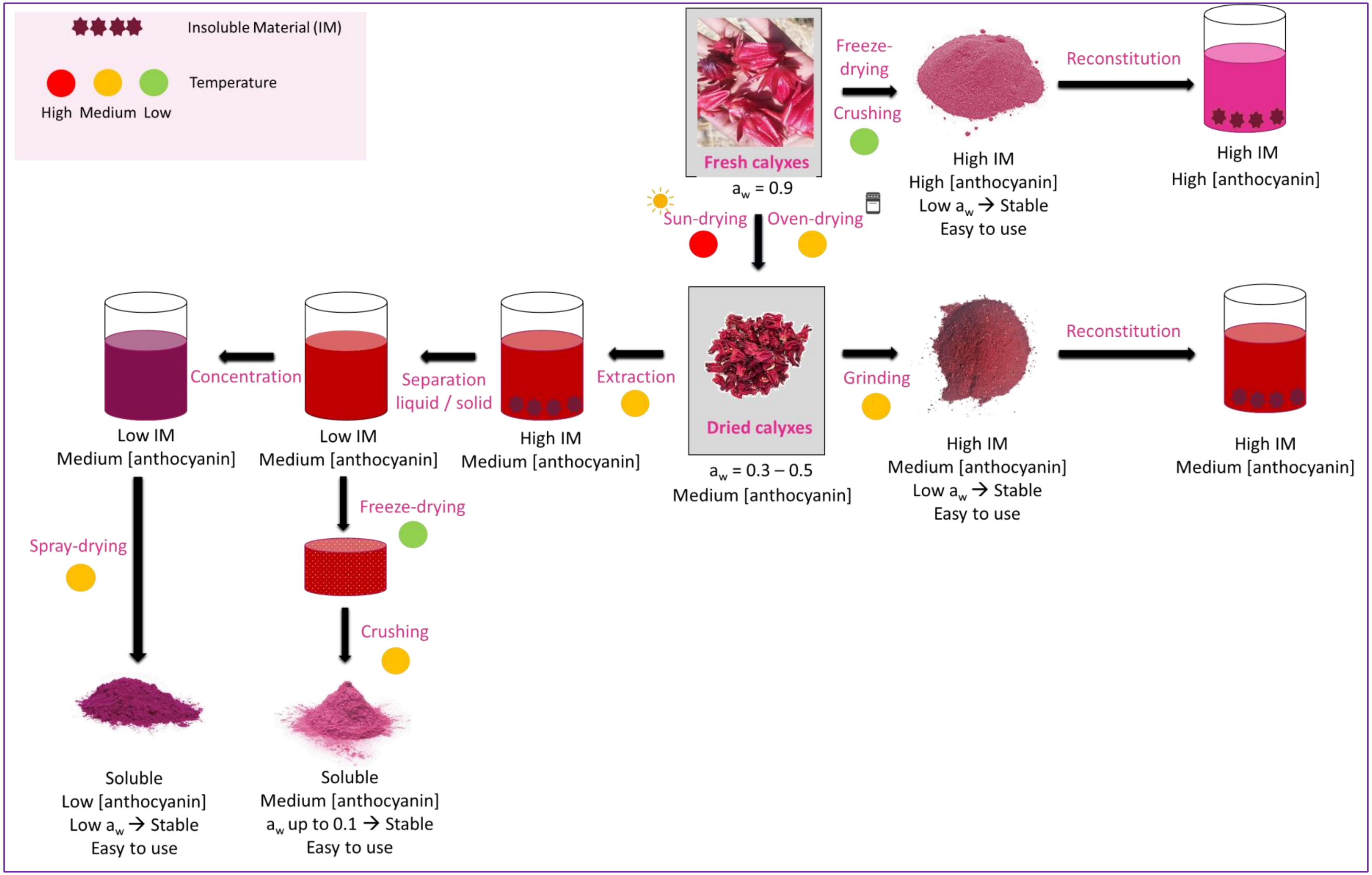


Figure 19: Impact of unit operations for hibiscus powder production, product stabilization and anthocyanin degradation.

2.4. Powder properties

Powder properties are often divided into three broad categories (Bhandari, 2013): fundamental, functional, and defective properties (**Figure 20**). The latter are properties that often limit their use for specific application and may not be desirable. Fundamental properties are the physical and chemical characteristics inherent in powders, and influence functional properties. These functional properties are related to the applications of powders as products or ingredients. These include the powder flowability and reconstitution properties (Bhandari, 2013; Masters, 2002).

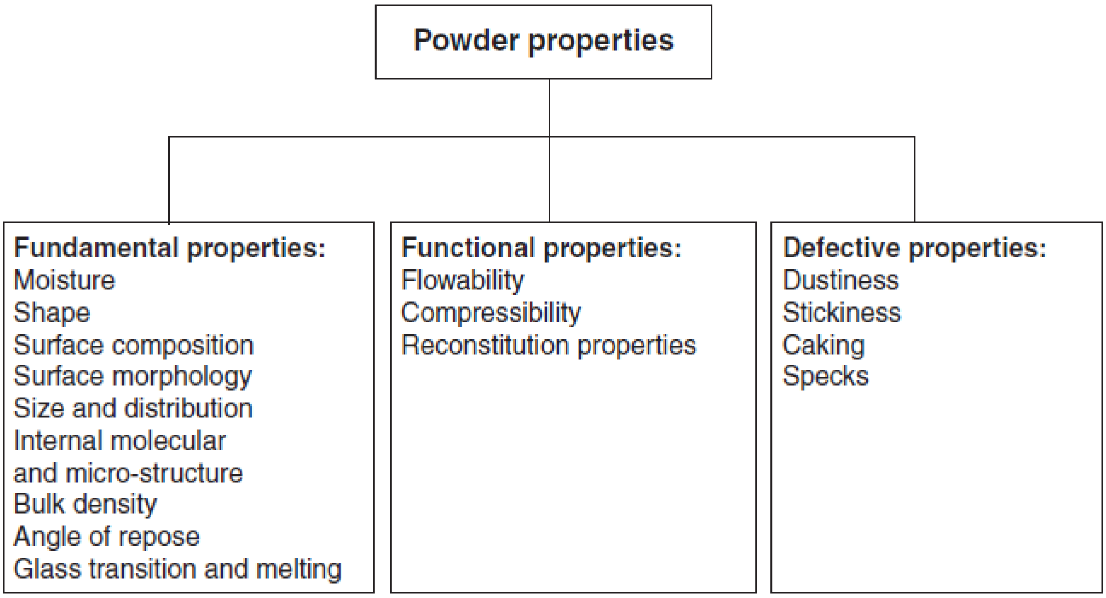


Figure 20: Three classes of powder properties (Bhandari, 2013).

2.4.1. Parameters influencing powder flowability

Powder flowability is defined as the relative movement of neighboring particles with each other or along the recipient wall surface (Petit et al., 2017). The powder flowability is function of the powder interactions with its environment and interactions between particles themselves (Petit et al., 2017). Good powder flowability means that powder flows freely when it is poured (**Figure 21**). On the contrary, powders characterized by poor or mediocre flowability, flow with difficulty for example cohesive powders. Upon powder production, storage and handling (discharging, weighing, blending, compression), powder flow properties are continuously modified by various thermal, mechanical and environmental stresses. It is therefore suitable to handle them under the best conditions, considering the majority of the parameters influencing their flowability to achieve the desired flow properties defined according to the final product characteristics. This also allows optimizing the production time, to make the processes profitable by limiting the product return by consumers.

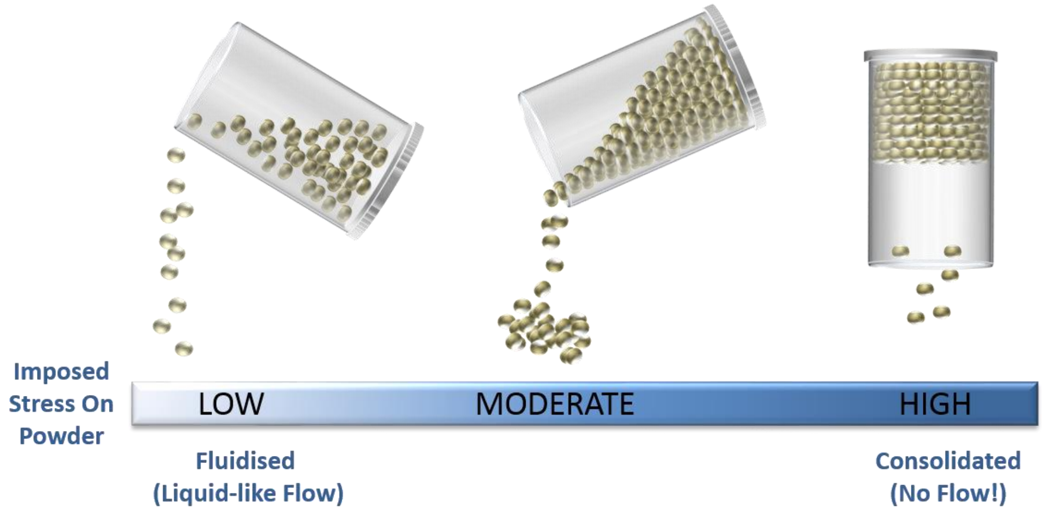


Figure 21: Powder flowability examples (freeman technology website).

Flowability depends on powder extrinsic factors as vibration, mechanical stresses and environmental conditions (temperature, humidity) and intrinsic factors including chemical composition, physical properties of powder but also of particle (particle size, shape, and roughness, powder density), that affect the powder interparticle forces (Figure 22). By studying the latter properties, authors succeed in evidencing the additional significant role of surface properties of food powders particle in driving their functional properties (Fournaise et al., 2021a; Kim et al., 2005; Tomas and Kleinschmidt, 2009; Xu et al., 2009). Indeed, particles in powders interact with each other or with the surrounding media through their surfaces.

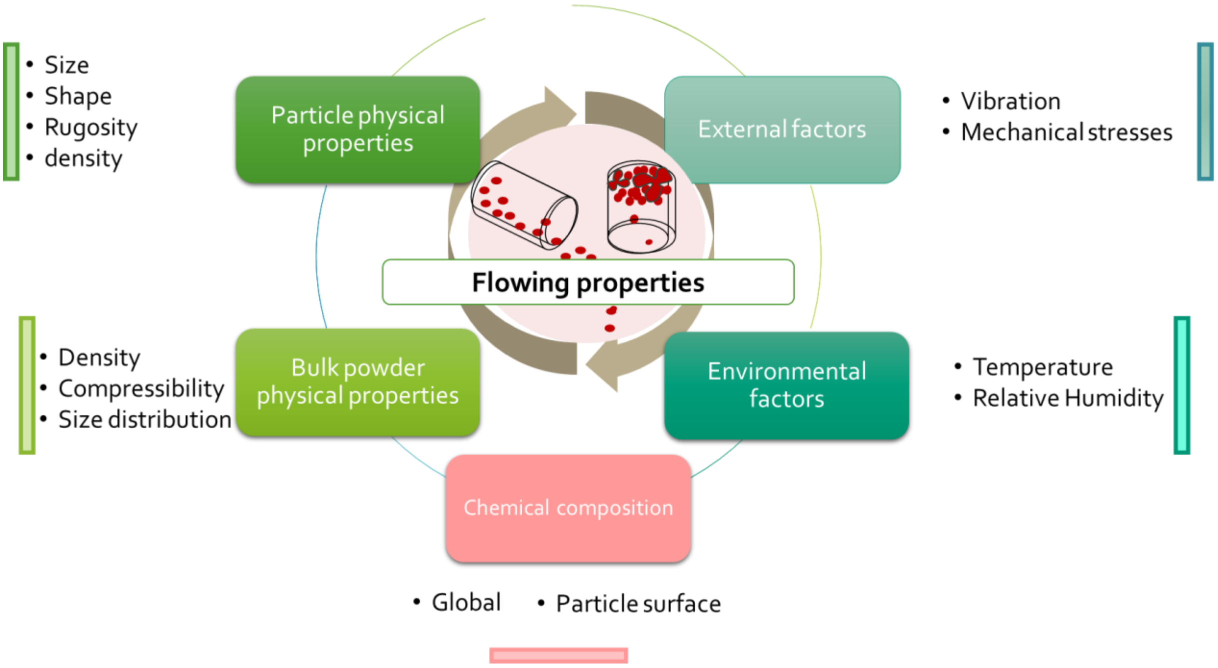


Figure 22: Main properties influencing the food powder flowability adapted from Petit et al. (2017).

2.4.1.1. Chemical composition

The particle surface composition is a key parameter to explain powder flowability. As flowability is the result of interparticle movements, the particle surface composition is one of the most important parameters to understand the flow of a powder. The particle surface composition varies from one food matrix to another but can also depend on the processing method applied. Indeed, during processing, a redistribution of the chemical compounds in the particles can occur. For example, Fyfe et al. (2011) reported that milk powder particles produced by pilot and laboratory dryers had lower fat content and higher protein content on surface than commercially produced powders. This difference is probably due to the difference in solids concentration of the feed used, neglecting the influence of the type of sprayers used. Water, lipids, proteins, and carbohydrates at the particle surface all impact the interparticle adhesion and consequently the powder flowability (Bhandari, 2013; Deli et al., 2019b; Fournaise et al., 2020; Kim et al., 2005). These macromolecules could form non-covalent bonds or chemical hydrogen bonds resulting in higher interparticle forces, consequently more cohesion (Bhandari, 2013) and possible undesirable aggregation or even caking.

2.4.1.2. *Physical parameters: particle size distribution and shape*

Particle size is one of the physical properties that has a predominant impact on powder flowability (Petit et al., 2017). Indeed, the particle size reduction reduces the distance between particles, and increases the contact area (Figure 23). This leads to an amplification of interparticle forces and results in a cohesion increase between particles. This observation is applied to different powder types including sugar (powdered and crystal sugar), milk, vegetable, tuber, plantain powders (Fitzpatrick et al., 2004; Fournaise et al., 2020; Gnagne et al., 2017; Irie et al., 2021; M'be et al., 2022; Petit et al., 2017; Rubel et al., 2018). Powders, when they are cohesive, flow with difficulty (poor flowability) because particles agglomerate or even cake. Caking is defined as the transition from a free-flowing powder into a solid block (Hartmann and Palzer, 2011; Zafar et al., 2017). Powder flowability depends also on its particle size distribution. When powders are heterogeneous for example, fine particles can adsorb at the surface of larger ones increasing the risk of rough particle formation that consequently induces frictional forces (Petit et al., 2017). The presence of fines can have a double effect and conversely induce a kind of ball-bearing effect, thus reducing the contact points between particles, leading to a reduction in frictional forces between particles and therefore a better powder flowability (Gnagne et al., 2017; Xie et al., 2021).

Another physical parameter influencing the powder flowability is morphology. Powder particle shapes are depending on the processing method (spray-drying, grinding, sieving...). An increase in cohesion limiting powder flowability is the result of high interparticle contact surfaces, which is favored by the presence of elongated shapes (fibers, needles, and rods), irregular shapes, and rough particles in powders. Indeed, these particle shapes strengthen interparticle bonds. Conversely, better flowability could be observed for powders with spherical particles because such shapes limit contact points, hence interparticle forces (Gnagne et al., 2017; M'be et al., 2022; Petit et al., 2017). Since food matrices are not perfectly homogeneous systems, the powder flow properties are therefore the result of combined effect of several powder particle characteristics that are required to be considered.

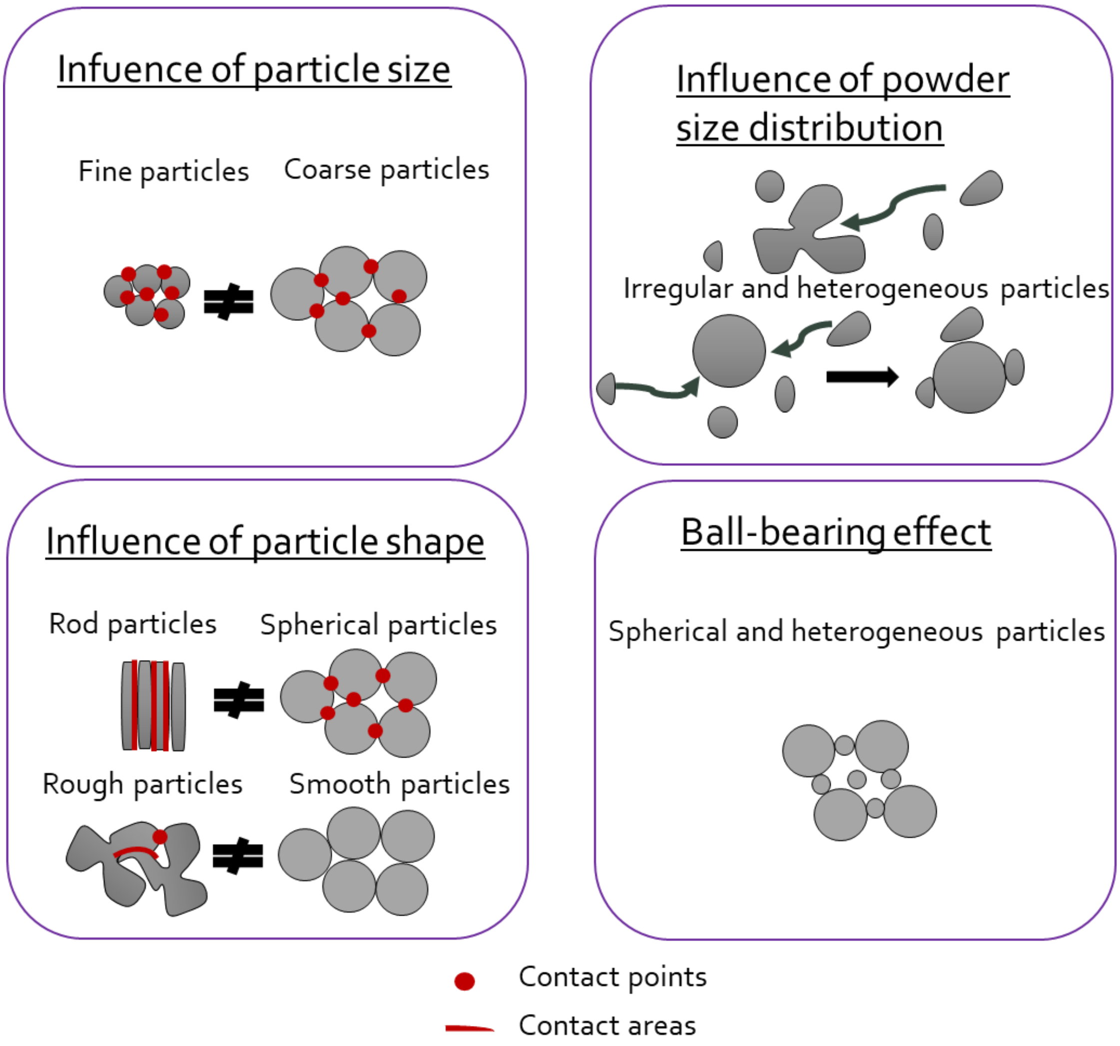


Figure 23: Influence of particle physical properties on interparticular interactions.

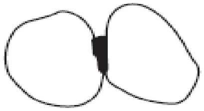
2.4.1.3. Structure

Food powders are composed of crystalline or amorphous particles, or a mixture of these two particle types. In the crystalline structural organization, particles are characterized by an ordered and very tight molecular arrangement. This is the case of salt, sugar and organic acids. Conversely, the molecules of amorphous particles are disordered, porous (Bhandari, 2013; Schuck et al., 2012b) and present some sites that favor external interactions especially those leading to water uptake and resulting in more interparticle cohesion. This consequently causes poor powder flowability. Amorphous powders include powders rich in polymers such as milk, fruit, honey and hydrolyzed protein powders. Therefore, the chemical composition, especially the polymer richness has a predominant influence on the structural organization of the powder (Seerangurayar et al., 2019).

2.4.1.4. Interparticle forces

The interparticle forces including liquid and solid bridges, van der Waals forces and electrostatic forces are the result of interparticle interactions (Table 2). These forces are function of the physicochemical characteristics of the particles (size, shape, chemical composition). In the case of powders rich in water, liquid bridges can form, linking the particles to each other by capillary forces and surface tension. These liquid bridges can become solid during storage or transformation processing as drying, because of liquid crystallization (formation of a crystalline structure) or glass transition (change of particle solid state into rubbery state). Interparticle bridges, whether liquid or solid, promote particle cohesion that prevents powders from flowing freely. In addition, particle cohesion and agglomeration could be induced by van der Waals forces (weak electrical interaction) (Feng and Hays, 2003) between fine powder particles, or the result of electrostatic forces due to friction between particles themselves and/or particles and the recipient wall containing the powders. These electrostatic forces are due to excess electrons of some particles aligning with electron-poor particles (of opposite charges) to balance particle surface charge (Bhandari, 2013; Machowski and Balachandran, 1998).

Table 2: Various interparticle interaction possibilities in powders (Bhandari, 2013).

Interparticle forces	Illustrations	Properties
Liquid bridges		Surface dissolution of glass transition and melting and cohesion, weak cohesion can break the bonds during handling and processing
Solid bridges		Glass transition, melting and fusion, strong force, stable
Van der Waals forces		Interactions of particle sizes of less than a micron
Electrostatic forces		Particle surface charge interactions
Mechanical interlocking		Irregular surfaces interlock each other, weak forces but physically locked from separation

2.4.1.5. Mechanical interlocking

Mechanical interlocking refers to a repositioning of particles after compression (Bhandari, 2013). This interlocking is favored for irregularly shaped, heterogeneous particles, particles with rough surfaces. It is also a function of external factors such as compression level, temperature, relative humidity (Bhandari, 2013; Özkan et al., 2002). Increasing these factors amplifies the interlocking level of particles.

2.4.1.6. Temperature

Temperature variation can induce, depending on the powder chemical composition, different phenomena including melting and glass transition.

On the one hand, lipid-rich powders subjected to any temperature above their melting point become sticky, leading to a strengthening of interparticle forces, which impairs the powder flowing.

On the other hand, polymer-rich powders, when subjected to certain temperature conditions, undergo the glass transition phenomenon. Glass transition refers to the transition of an amorphous solid from its glassy state (the material is characterized by a high internal viscosity) to a rubbery state (the material viscosity decreases) (Roos, 2002; Schuck et al., 2012b). This transition to the rubbery state generates an increase in the particle surface energy that leads to particle mobility and interaction between particles, which can adhere to each other (Bhandari, 2013; Bhargava and Jelen, 1996; Schuck et al., 2012b). In this sense, the glass transition temperature must be higher the powder temperature to avoid particle cohesion but also to favor a brittle particle, easier to grind for powdering. Therefore, the glass transition temperature is an essential parameter for controlling powder flowability.

The glass transition is depending on the proportion of amorphous particles, temperature, water content, and chemical composition of powders (Bhandari, 2013; Fitzpatrick et al., 2007; Özkan et al., 2002). Increasing the temperature and/or air relative humidity (Figure 24) during processing (spray-drying, grinding) or storage favors the powder glass transition (especially those rich in sugar) which then form aggregates or cake preventing their good flowability.

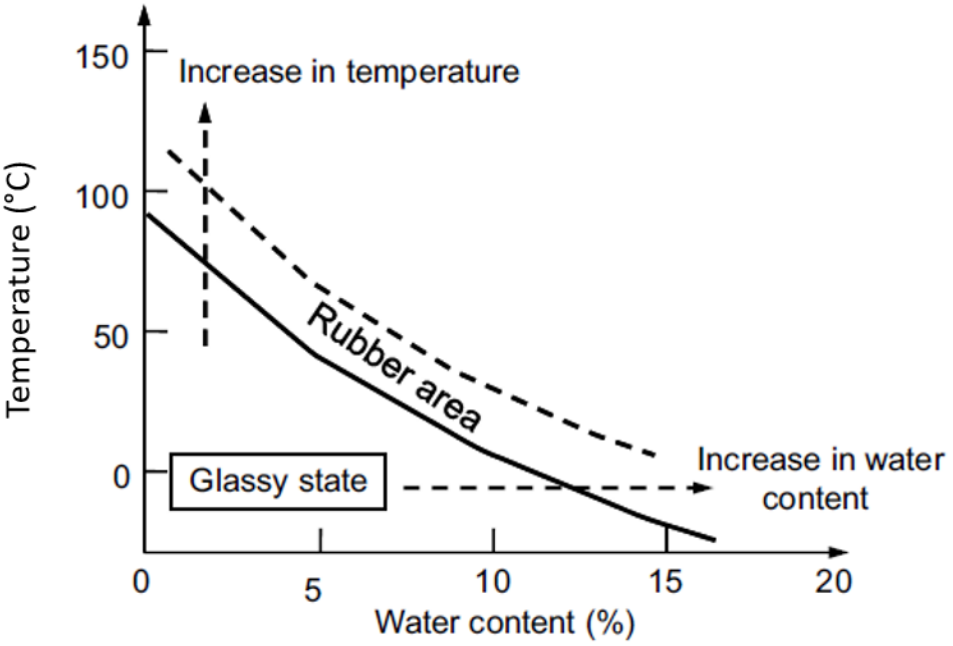


Figure 24: Glass transition as a function of temperature and water content (Roos, 2002; Schuck et al., 2012b).

When the particle water content increases, the glass transition temperature decreases leading to the material viscosity reduction that results in plasticizer effect of water on powders (Aguilera et al., 1995). As result, water can promote more interparticle forces and inhibit the good powder flowability. Sugars has also a dominant effect on glass transition, as demonstrated Shrestha et al. (2007) in their study about the influence of protein and lactose. In addition, powders such as fruit juice powders characterized by their richness in low molecular weight sugars are very hygroscopic so that they present low glass transition temperature. They must be manufactured or stored in controlled conditions to alleviate the glass transition issue and maintain their initial flowability.

2.4.2. Parameters influencing powder reconstitution

Reconstitution is an essential step for the employability of powders. Hibiscus powder, for example, is reconstituted with water to produce beverages, infusions or to enhance the color of preparations. This reconstitution step allowing the extraction of soluble molecules is essential to their use. Therefore, the optimization of beverage production, time or cost of extraction unit operations, inevitably requires the control of biomolecule extraction (Fang and

Bhandari, 2011; Saggin and Coupland, 2002; Selomulya and Fang, 2013). Powder reconstitution occurs in different stages: wetting, sinking/swelling, dispersion, and solubilization (Fang et al., 2008) (**Figure 25**).

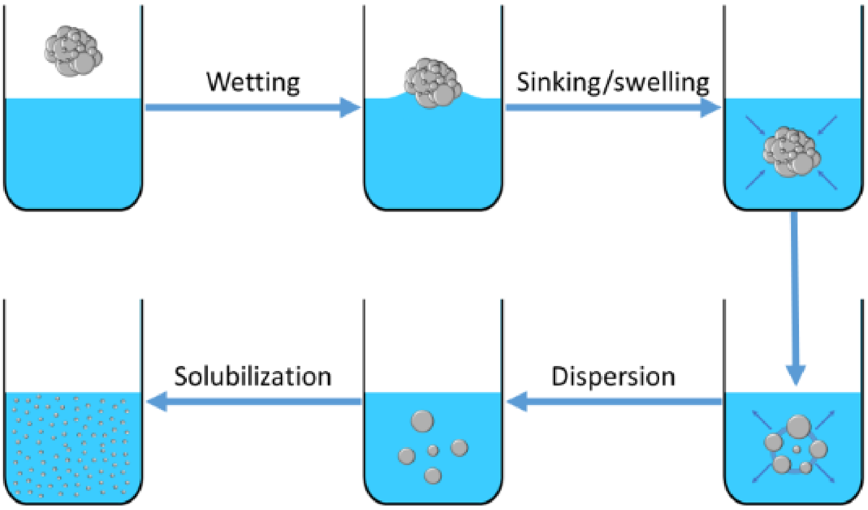


Figure 25: Powder reconstitution steps (Forny et al., 2011; Fournaise, 2022).

2.4.2.1. Wetting

Wetting is the phenomenon during which the liquid gradually replaces the gas phase at the particle surface after that the liquid was put into contact with particle surface (Forny et al., 2011; Fournaise et al., 2020; Selomulya and Fang, 2013). Wettability is a reconstitution property that can be assessed by measuring the time (s) required for a quantity of powder to penetrate through the free surface of the liquid at rest. For example, date powder wettability ranges from 13 - 27 s (Seeranguryar et al., 2018), that of commercial milk powders generally ranges from 24 s for skim milk powders to 120 s for whole milk powders (Fournaise et al., 2020; Schuck et al., 2012b). The short wetting time of date powders is due to their richness in carbohydrates that are hydrophilic molecules able to enhance wetting in contrast to lipids found at the surface of whole milk powder particles.

From a physical point of view, large particles due to their smaller contact angles (θ, angle between liquid and solid surfaces, **Figure 26**) are more wettable than small particles (Kirchberg et al., 2011; Schuck et al., 2012b) (**Figure 27**). Similarly, irregular and surface-roughened particles yielding high contact angle impair the wetting step (Kirchberg et al., 2011; Nguyen et al., 2021).

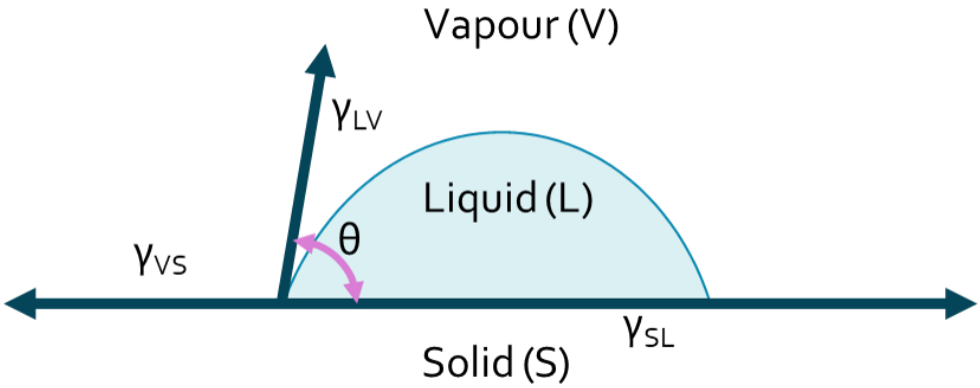


Figure 26: Determination of angle contact, γ surface tension (N.m^{-1}).

The relationship between the contact angle and the surface tensions is defined according to equation 4.

$$\gamma_{SL} = \gamma_{VS} - \gamma_{VL} * \cos\theta \qquad (4)$$

With:

- Υ_{SL} : the solid liquid surface tension (N.m^{-1}),
- Υ_{VS} : the vapor solid surface tension (N.m^{-1}),
- Υ_{VL} : the vapor liquid surface tension (N.m^{-1}).

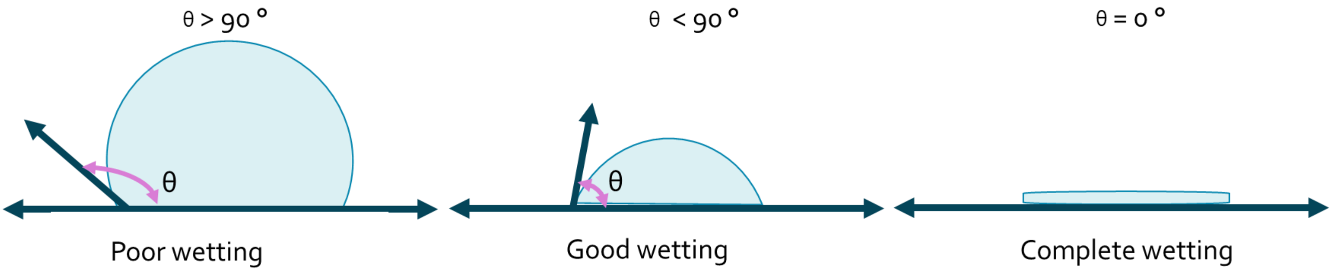


Figure 27: Wetting evolution in function on contact angle.

2.4.2.2. Sinking and swelling

Sinking is the phenomenon during which water diffuses into the particle, increasing its density, which enables the particle to overcome the surface tension at the particle-liquid interface (Burgain et al., 2016; Fournaise et al., 2022a; Gaiani, 2006). The sinking step also depends on the particle surface composition. Indeed, a particle surface mainly composed of hydrophobic compounds such as fat limits and slows down the sinking step of the powder in water (Fang et al., 2008).

Simultaneously, the liquid penetrates the particles and results in an increase in particle size; it is the swelling step. Swelling is also marked by a local increase in liquid viscosity around the particle which would indicate a softening of particle surfaces (De Richter et al., 2022; Fournaise et al., 2022a; Kumar et al., 2018; Mitchell et al., 2019; Sweijen et al., 2017). Swelling depends on the particle chemical composition but also the particle size. Indeed, particles swell rapidly for powders rich in polymers such as proteins, carbohydrates, fibers unlike powders rich in lipids (Cuissinat and Navard, 2008; Forny et al., 2011; Yang et al., 2016). Moreover, in their studies on several powders, Fournaise et al. (2022) demonstrated that the swelling time lengthens for powders made of large particles, due to their reduced specific surface area.

2.4.2.3. Dispersion

The dispersion step is marked by a division of parent particles (agglomerated or not) into several daughter particles (Selomulya and Fang, 2013). This step takes place when the interparticle forces (liquid and solid bridges, hydrogen or intraparticle bonds, van der Waals interactions) are broken. It depends on the affinity degree between the solvent and particles, the powder chemical composition, but also the particle size distribution (Cuq et al., 2011; Fournaise et al., 2022b; Freudig et al., 1999). In an aqueous medium, for example, a particle with a hydrophilic surface is more likely to disperse easily than a hydrophobic particle (Fournaise et al., 2022a). Dispersibility is assessed by the amount of dry matter dispersed in water that can pass through a sieve of defined mesh diameter. This value expressed in percentage is the dispersibility index (IDF, 1979). The dispersibility index of ground hibiscus calyx powder (<180 µm) is roughly 67 %, lower than that of milk powder 90 – 95 % (Bhandari Bhesh et al., 2013; Deli et al., 2019b; Schuck et al., 2012b). This difference can be explained by the hibiscus powder heterogeneity, the chemical composition differences, and native cell structure differences between milk and hibiscus powders. Hibiscus powder cell structure is likely more resistant to the water osmotic pressure than that of milk powder. Accordingly, this sensitivity probably makes milk powder more dispersible.

2.4.2.4. Solubilization

This step corresponds to the disappearing of particle granular structure after complete solubilization of powder. It is related to solubility, which is the ability of powders to rehydrate under predefined conditions. This property depends on the particle chemical composition and the physical characteristics. Indeed, studies have shown good hygroscopicity and solubilization for powders rich in carbohydrates or low molecular weight proteins (Bhandari Bhesh et al., 2013; Fournaise et al., 2022b). On the other hand, solubilization can be slowed down for powders rich in insoluble components (e.g. fibers), or crystalline structures. In the latter case, the tight structure of molecules hinders water absorption through the particles. Powder solubility can be improved by limiting insoluble material during the manufacturing process (Bhandari Bhesh et al., 2013; Schuck et al., 2012b). In addition, solubility is accelerated for smaller particle sizes due to their great specific surface area (Fournaise et al., 2022a).

To sum up, each reconstitution step is dependent on powder intrinsic characteristics, such as chemical composition (water content, carbohydrates, lipids, proteins, fibers, minerals), physical properties (porosity, particle size distribution, particle shape and structure, capillarity), and solvent-particle interaction (Deli et al., 2019b; Gaiani, 2006; Selomulya and Fang, 2013; Tan and Sulaiman, 2020). All of these features are related to the material and the powder production processes and they can also evolve depending on their surrounding environment (Figure 28): for example, powders stored at elevated relative humidity tend to agglomerate due to water adsorption to surface and the establishment of liquid then solid bridges. When considering powder reconstitution, extrinsic factors such as the nature of the solvent, temperature, and stirring conditions are of paramount importance (Gaudel et al., 2022; Gonzalez-Palomares et al., 2009; Osman and Endut, 2009a). Under high stirring speed and temperature conditions, reconstitution rate can be improved, including sinkability and dispersibility (Bhandari Bhesh et al., 2013; Gaudel et al., 2022; Mitchell et al., 2015). This increase in reconstitution rate is on one side due to the increase in shear forces induced by stirring. On the other hand, the impact of temperature is explained by a change in the particle structure and composition that facilitates the penetration of the liquid.

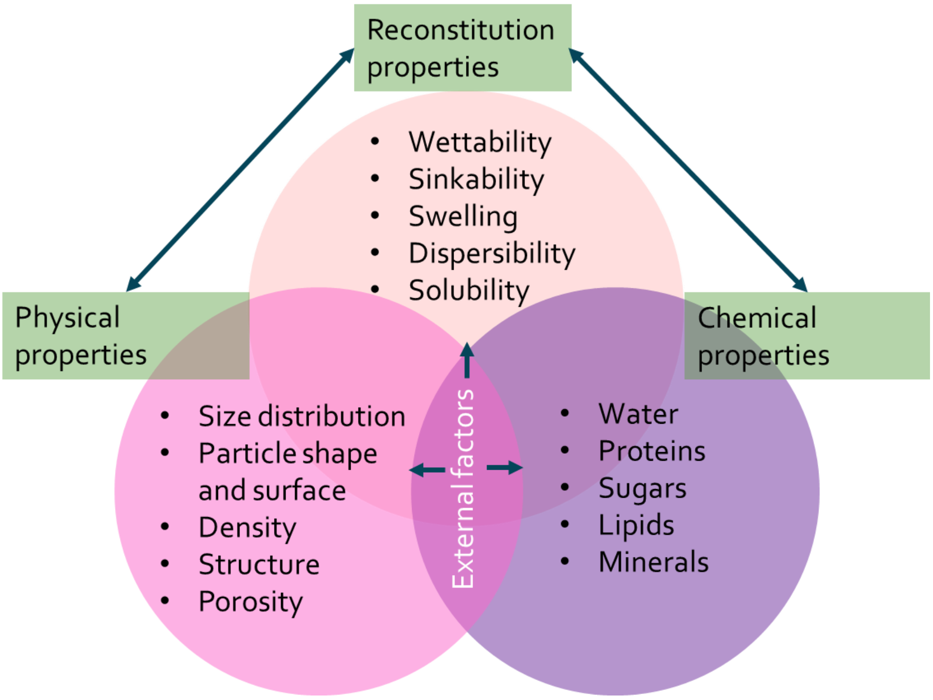


Figure 28: Interactions between reconstitution and physicochemical properties of powders.

2.5. Conclusion

Hibiscus calyxes are edible vegetables with attractive natural colorings, and presenting interesting health and medicinal benefits. The challenge for food industry is to be able to respond adequately to consumer demands, reduce food waste, allow availability over a long time, and make the product accessible to all interested parties throughout the world. To this end, several unit operations have been investigated and successful results have been obtained. Drying is very useful to overcome the issue of seasonality, facilitates transport and thus allows worldwide access to this tropical plant. Complementary methods to drying, such as powder production, have further facilitated the use of hibiscus calyxes by increasing the product specific surface area. This improves therefore the availability of anthocyanins and polyphenols, the antioxidant activity that is the key indicator of the hibiscus calyx health benefits. However, the main barrier to the application of these processes is the thermal sensitivity of anthocyanins, which depends on the parameters of the different processes. Freeze-drying although expensive is the least harmful drying method for anthocyanins. Spray-drying and freeze-drying/grinding must necessarily be combined with pretreatments (hibiscus drying, extraction, filtration, concentration) which result in cumulative losses of anthocyanins and polyphenols. In this sense, drying coupled with grinding would presents the best advantages to produce hibiscus powder since there are only two unit operations to master for a good biomolecule preservation. The additional advantage is that this method allows limiting the waste issue induced by the extraction/filtration steps, by using the entire calyx. Given the grinding step produces heterogeneous powder particles, it is also very important to consider all the physicochemical properties (particle size and shape, structure, porosity, composition…) as they have an impact on powder functional properties (flowability, reconstitutability). Indeed, poor powder flowability is sensitive to undesirable particle agglomeration and powder caking, which results in a decrease in process performance and in product acceptance. In addition, the mastering of the reconstitution step is a key factor in optimizing the soluble material extraction including biomolecules with the aim to obtain hibiscus extracts or drinks.

Chapitre 3 : Materials and methods

3.1. Materials

3.1.1. Plant materials

First calyx harvest. *Hibiscus sabdariffa* flowers from Ganaoni (9° 17′5.935′′N 6° 19′ 22.262′′W, Bagoue region), northern Ivory Coast, were collected in January 2019 and shelled to remove seeds and stems (Figure 29). Foreign objects were removed, and then the calyxes were pre-treated by exposure directly to the sun (at about 37 °C air temperature) for at least 7 days until reaching a water content of 18.50 g / 100 g on a wet basis. Sun-dried *Hibiscus sabdariffa* calyxes were finally packed in sealed bags and stored at 4 °C.

Second calyx harvest. *Hibiscus sabdariffa* calyxes were collected in December 2021 from Sinematiali (9° 34' 59.999" N 5° 22' 59.999" W), a city in the north of Ivory Coast.

Figure 29: Harvesting and shelling of *Hibiscus sabdariffa* calyxes.

3.1.2. Chemicals

Potassium chloride, gallic acid, methanol, 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate were purchased from Sigma-Aldrich (United States of America). Sodium acetate and hydrochloric acid were obtained from VWR Prolabo chemicals (Belgium) and Carl Roth (Germany), respectively. Acetonitrile and formic acid were purchased from Carlo Erba (United States of America).

3.2. Powder production methods

3.2.1. Powders from pre-dried *Hibiscus sabdariffa*

The global powder production process is presented in Figure 30.

Oven drying. Three batches of 900 g sun-dried calyxes were weighed and sorted. One of these batches was considered as the reference (T0) and was not subjected to oven drying. Two batches were dried in an oven (Memmert, ULM 400, Schwabach, Germany) at 45 °C for 1 h (T1) and 2 h (T2) to further reduce the water content. The oven was preheated before introducing a perforated tray (1 cm holes) covered by a 1.5 cm thick calyx layer. The air velocity was assumed to be constant. After the drying process, the dried calyxes were preserved in a desiccator before grinding.

Grinding. First, calyxes were pre-ground in a blender (Moulinex, DPA1, Lourdes, France) for 5 s and then ground (in less than 1 min) in an Ultra Centrifugal Mill ZM 200 (Haan, Germany) supplied with a 24-tooth rotor of 99 mm diameter, a stator consisting of a stainless steel ring sieve composed of 1 mm diameter trapezoid holes, and a < 1 cm feed hopper. Each batch of 900 g calyxes was divided into 3 batches of 300 g which were milled respectively at 10 000, 12 000, and 14 000 rpm (corresponding samples were respectively named B10, B12, and B14). Temperatures of all grinding-induced powders were measured to check that powders were not significantly heated. They ranged from 25 °C to 30 °C. Powders were packed in sealed bags and stored at 4 °C sheltered from light until analysis.

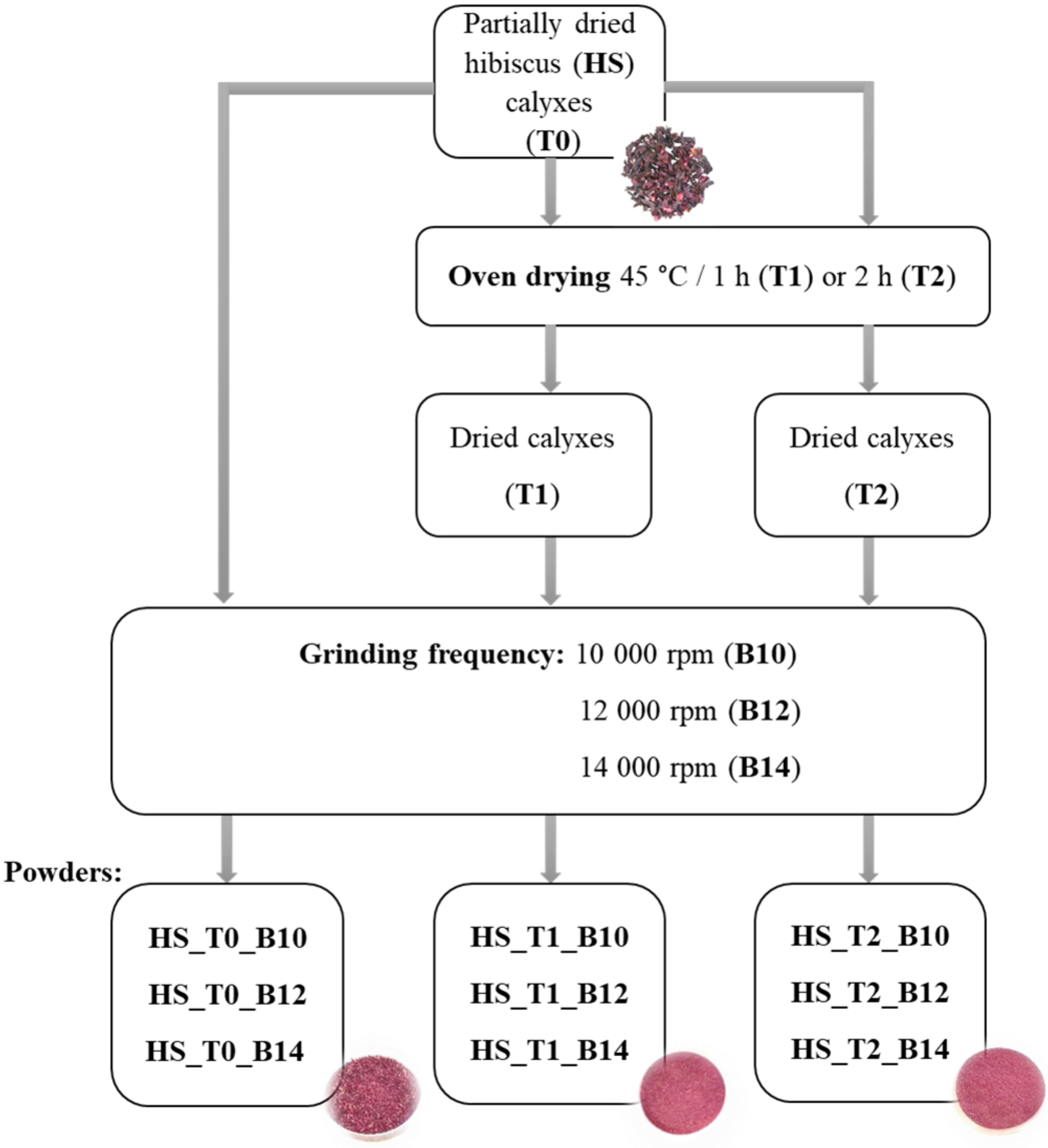


Figure 30: Manufacturing process of pre-treated *Hibiscus sabdariffa* calyx powders.

3.2.2. Powders from fresh *Hibiscus sabdariffa* (second harvest)

Calyxes were dried directly after harvesting by two methods to reach a water content of 10 g / 100 g product. Controlled drying at 55 °C, using an oven-dryer (Klarstein, Germany), with 100 % ventilation and a duration of 24 h was carried out on a part of the fresh calyxes. The calyxes were spread in a single layer on the perforated racks (0.7 cm × 0.7 cm) of the oven. The second batch of fresh calyxes was subjected to a traditional drying (solar-drying) during 3 days.

The calyxes were spread in a stainless aluminum tray, and exposed to sunlight at an average daily temperature of 31 ± 1 °C.

The dried calyxes were ground at 14 000 rpm (Centrifugal Mill ZM 200, Haan, Germany) to be converted into powder (Figure 31).

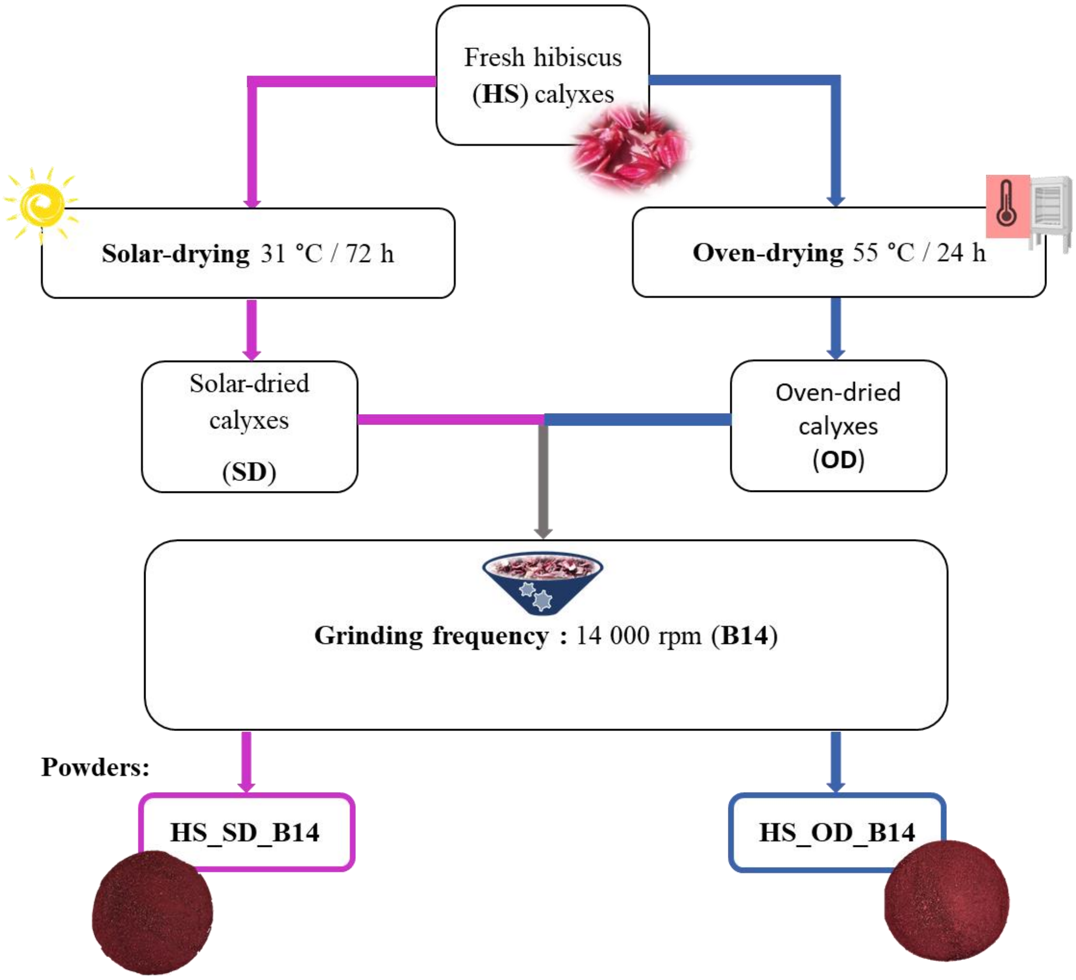


Figure 31: Manufacturing process of fresh *Hibiscus sabdariffa* calyx powders by oven-drying or solar-drying followed by a grinding step.

3.2.3. Powder fractionation

Powder fractionation according to particle size was performed to better understand its influence on powder flowability and reconstitution. The fractionation was performed with an Analysette 3 Spartan vibrating sieve shaker (Fritsch, Idar-Oberstein, Germany). The choice of sieve mesh sizes was based on the particle size distribution of the three initial powders, which allow separating the two main populations of every powder. Thus, 200 - 300 g of powder samples were poured on a sieve with a 212-µm mesh (212-µm mesh, chosen according to the particle size distribution) mounted on the sieve shaker. Sieving was carried out by applying vertical oscillations of the sieve for 15 min at an amplitude of 1 mm. The 49 - 50 % passing fraction was called fine powders (F) and particles retained by the sieve were designated as coarse powders (C) (Figure 32).

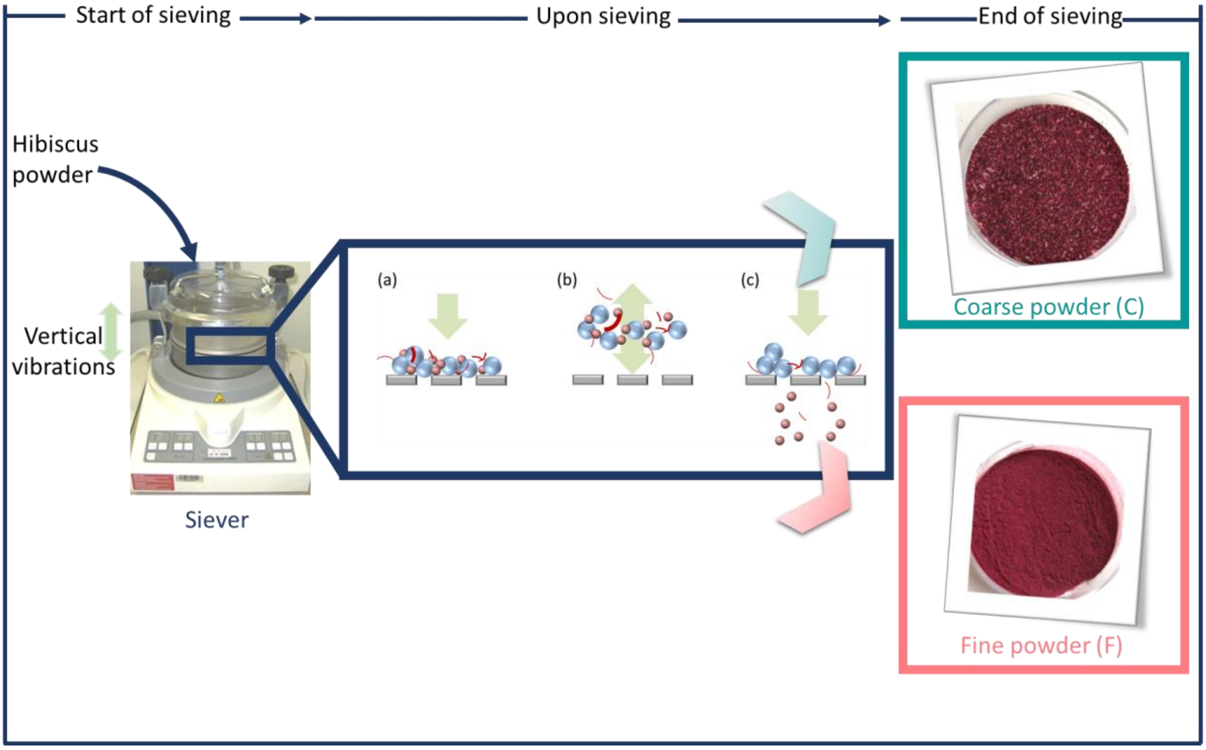


Figure 32: Fractionation process by sieving applying vertical vibrations; a - initial deposition of particles on the sieve, b - vertical vibration allowing particle individualisation, c - passing of the smaller particles through the sieve; adapted from Yoon et al. (2016).

3.3. Powder physicochemical characterization

3.3.1. Water content and water activity

The water content was determined by the loss of water mass of 2.5 g powder for at least 36 h at 103 °C until a constant mass was obtained following ISO 5537: 2004 (AFNOR, 2004). Water activity was determined by using a HygroPalm portable water activity meter (ART NO:HC2-AW, ROTRONIC, France) at 20 °C. More precisely, a 15 g sample was introduced into a polypropylene cup that was deposited in the sealed enclosure of the apparatus. The free water moistens or dries the air inside the enclosure until reaching equilibrium.

3.3.2. Powder color measurements

Powder color was determined using a CR-400 chromameter (Konica Minolta). For each sample, 10 g of powder was poured into a petri dish, and color measurement was performed according to the CIE L*, a*, b* color space. The lightness (L*) ranges from 0 (black) to 100 (white); the red-green balance (a*), from -100 (green) to 100 (red); and the yellow-blue balance (b*), varying from -100 (blue) to 100 (yellow). From these parameters, other colorimetric parameters can be calculated including the chroma (C*) and the hue angle (H*) related to saturation and tint, which are defined in equations 2 and 5, respectively.

$$C^* = \sqrt{(a^{*2} + b^{*2})} \tag{2}$$

$$H^* = \arctan\,(b^*/a^*) \tag{5}$$

3.3.3. Particle size distribution

The Mastersizer 3000 equipped with the Aero S dry dispersion unit (Malvern Instrument, United Kingdom) was used to measure particle size by laser diffraction at 633 nm. About 5 g of powder was poured into the feed hopper and conveyed in the system using 30 % vibration. The measurements were performed three times at a dispersing pressure of 1.5 bar, a hopper gap of 4 mm, an obscuration comprised between 0.5 and 1.5 %, and a background calibration of 10 s. The size estimator was the median diameter (µm) in volume. Dispersion of particle sizes can be evaluated by measuring the span, which normalizes the width of the distribution relatively to the median value according to equation 6:

$$SPAN = (D_{90} - D_{10})\,/D_{50} \tag{6}$$

Where:

- D_{10} (µm): 10 % of the sample particles are smaller than this diameter,

- D_{50} (µm): 50 % of the sample particles are smaller than this diameter,
- D_{90} (µm): 90 % of the sample particles are smaller than this diameter.

3.3.4. Particle morphology

3.3.4.1. *Scanning electron microscopy*

A scanning electron microscope (Hitachi S-4800, Japan) operating at 1 kV was used to observe particle morphology and polydispersity but also to characterize the surface of the hibiscus particles. Powders were deposited on a carbon tape. Samples were then metallized with a carbon metallizer. Topographic images were taken for each powder at 500 ×, 1 500 ×, and 3 000 × magnifications.

Particles from reconstituted powder were observed according to the following method. A 0.2 µm filter RC-membrane (PP-housing minisart RC 4, Sartorius Stedium Biotech, Germany) was used to vacuum filter powder solutions upon reconstitution at 20 °C. Retained particles were dried for 1 h at 103 °C, then observed at 500 x magnification under the same scanning electron microscope operating at 1 kV.

3.3.4.2. *Optical microscopy*

Particle morphology was examined using an optical microscope Olympus BH-2 (Japan) equipped with a 40 × magnification objective.

3.3.4.3. *Morphogranulometry*

Particle size and shape were characterized using a QICPIC dynamic image analysis system (OASIS/L dry dispersing system, Sympatec GmbH, Clausthal-Zellerfeld, Germany) (Figure 33). The camera captured images of the particles, which were analyzed with the PAQXOS 5.0.1 software. From the 3D particle, image analysis provides 2D particle image and assesses their size and shape parameters.

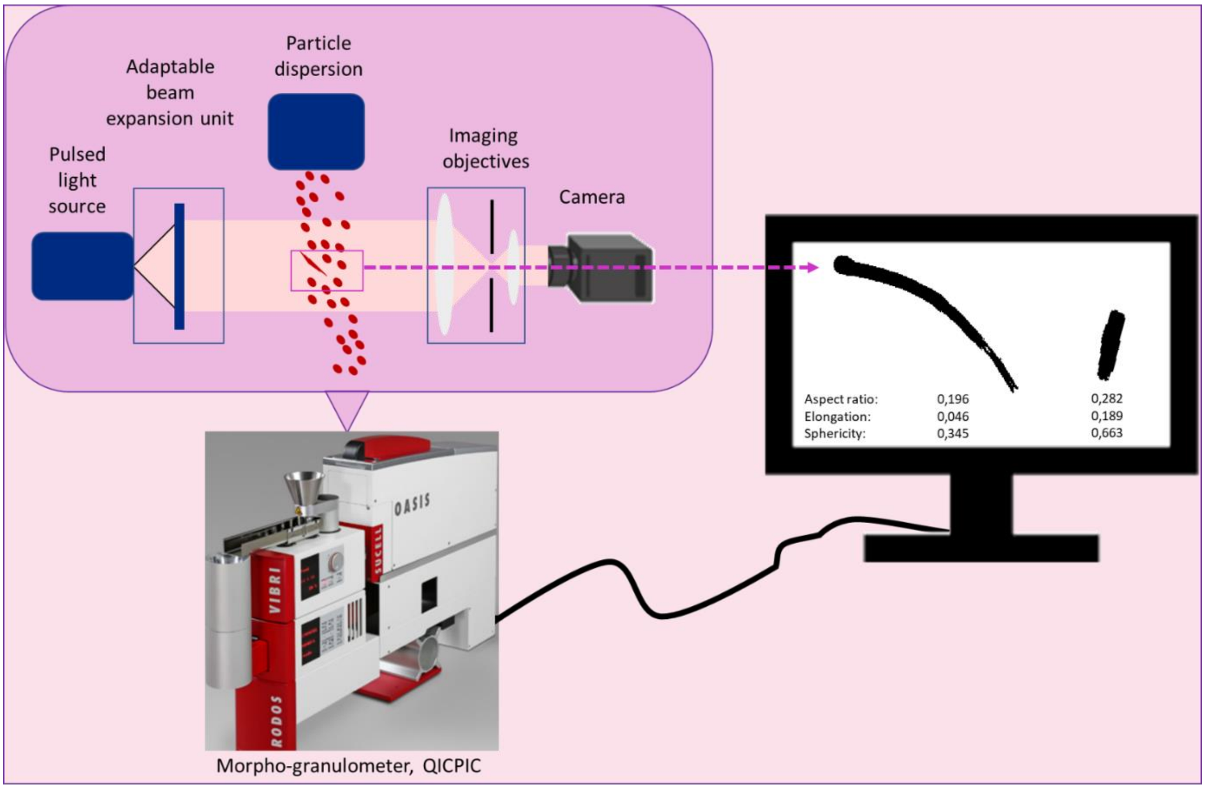


Figure 33: Qicpic morphogranulometer system using to analyse particle size and shape.

The QICPIC morphogranulometer was supplied with an OASIS/L dispersion system, which combines dry and liquid dispersers in one device. For powder analysis, the RODOS dry disperser was used. Roughly 1 g powder was introduced into a hopper set at 1.5 mm hopper gap conveyed by vibration (set at 30 %) using the VIBRI vibrating feeder, and dispersed in the system using compressed air at 0.5 bar. The M4 optical module (1.1 – 2 253 µm measuring range) was used.

The particle sizes are evaluated on the basis of the diameter of a circle that has the same area as the projection area of the particle (D_{EQPC}) (**Figure 34**).

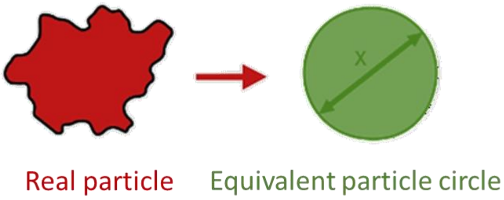


Figure 34: Representation of the principle of EQPC measurement : diameter of a circle of equal projection area (green) than of the real particle (red), adapted from Sympatec site.

Particle shape features such as elongation, and sphericity were determined.

Elongation (El) is a shape parameter used for fiber-shaped particles (**Figure 35**); it is defined by the ratio of the fiber width to the fiber length (equation 7). It is comprised between 0 and 1, this latter value corresponds to circular and square particles. The longer the fiber, the lower its elongation.

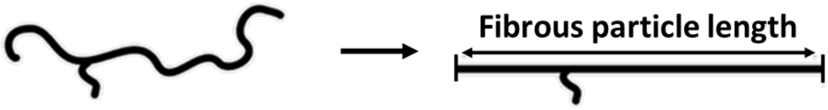


Figure 35: Representation of elongated fibrous particle, adapted from Sympatec website.

$$El = \ fiber\ width/fiber\ length \tag{7}$$

Aspect ratio (AR), ranging between 0 and 1, indicates the elongation and level of irregularity of the particles and is defined by equation 8.

$$AR = d_{Feret\ min}\ /d_{Feret\ max} \tag{8}$$

With:

- $d_{Feret\ min}$ (µm): minimal Feret diameter, where the Feret diameter designates the distance between the two parallel planes restricting the object perpendicular to the direction of particle size measurement (**Figure 36**),
- $d_{Feret\ max}$ (µm): maximal Feret diameter. There is more difference between the minimum and maximum Feret diameters of irregularly shaped particles.

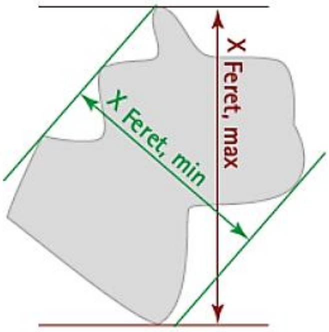


Figure 36: Definition of Feret diameters (x) from Sympatec website.

The sphericity (S) is defined in equation 9 and ranges from 0 to 1. The smaller the value, the more irregular the particle shape. A sphericity value of 1 corresponds to spherical particle.

$$S = P_{EQPC} / P_{real} \tag{9}$$

With:

- P_{EQPC} (µm): the perimeter of a circle with the same area as the projected surface of the particle,
- P_{real} (µm): the real particle perimeter.

3.3.4.4. Proportion of fibrous particles

Hibiscus powders present a high heterogeneity regarding particle size but also particle shape. Among them, fibrous particles play a major role on powder flowability and powder reconstitutability. For these reasons, the definition of morphogranulometric criteria to quantify fibrous particles is important for the description and the understanding of powder functional properties. Fibrous particles (**Figure 37**) were considered with low elongation, low aspect ratio and low sphericity values. Aspect ratio alone was not sufficient to discriminate fibrous particles, as low AR values were obtained for fibers but also for irregular rounded or elongated particles. When adding the criterion of low elongation, the identification of fibrous particles was better but some other particles were still retained by the software associated with the morphogranulometer. It was also necessary to filter only low sphericity particles to ensure a good quantification of fibrous particles.

The choice of adequate thresholds for these three shape factors for the discrimination of fibrous particles of hibiscus powders required many trials that are not developed here for concision purposes. In this work, fibrous particles were considered to have an elongation of less than 0.50, an aspect ratio inferior to 0.55, and a sphericity under 0.50. The proportion of fibers in 100 g powder (Fb) was calculated with equation 10.

$$Fb = (Fiber\ number / particle\ total\ number / powder\ mass) \times 100 \tag{10}$$

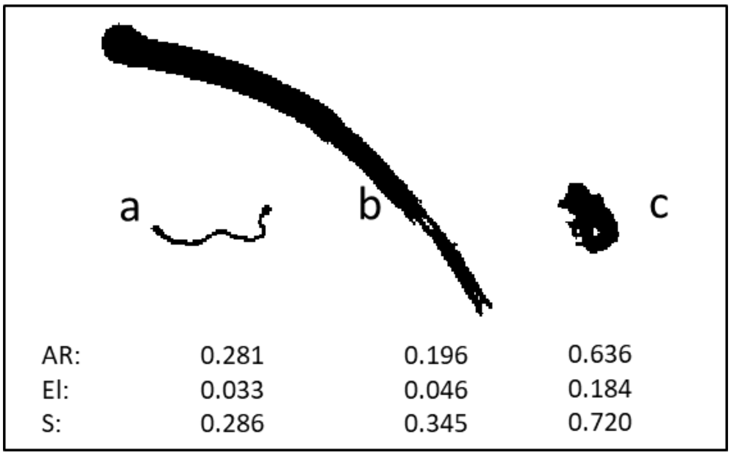


Figure 37: Particle images recorded during a morphogranulometric measurement and associated shape values. a,b examples of fibrous particles, c- non-fibrous particle, from the powder HS_T2_B14; AR - Aspect Ratio, El – Elongation, S - Sphericity.

3.3.5. Powder flowability

Powder flow behavior was analyzed using a FT4 powder rheometer (Freeman Technology, Worcestershire, United Kingdom) with 25 mm accessories (Figure 38).

Compressibility test. The compressibility test allows the measurement of the evolution of powder density as a function of the applied normal stress. To this end, three conditioning cycles were performed with a 23.5 mm blade followed by the split of the vessel (25 mm x 10 mL). The blade was replaced by a vented piston to subject the powder to nine levels of normal stress from 1 to 15 kPa. The normal stress was held constant for 60 s to allow the powder to stabilize. The compressibility test allows obtaining compressed bulk density (CBD, expressed in g.mL^{-1}), which is the ratio between the powder mass and the volume of the powder bed after compression (equation 11).

$$CBD = (powder\ mass\ after\ split/volume\ after\ compression) \tag{11}$$

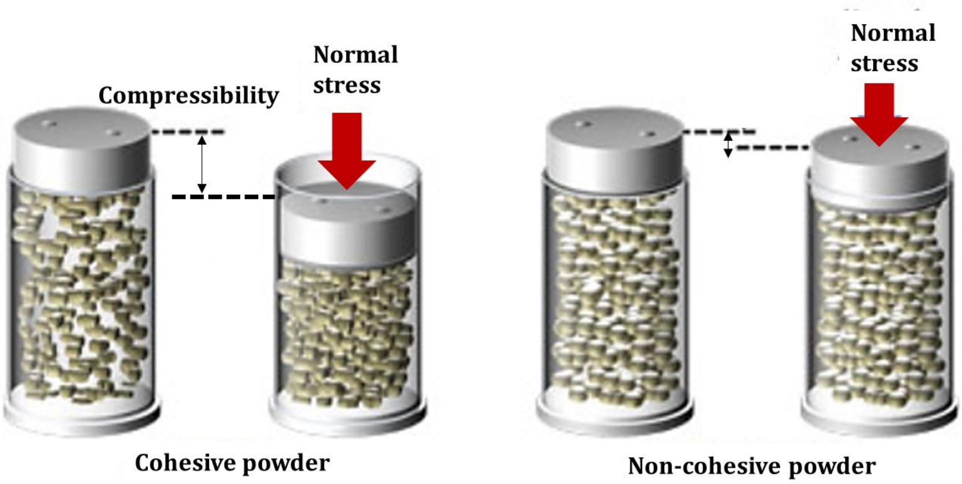


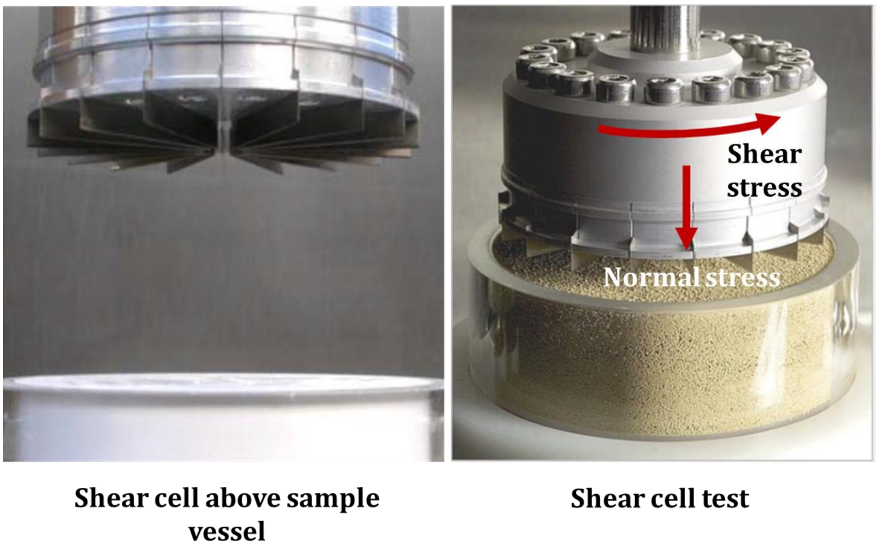
Figure 38: Compressibility tests using a FT4 powder rheometer, adapted from Freeman Technology website.

Shear cell test. The shear cell test is a method allowing to determine the incipient shear stress required to put a consolidated powder that was previously at rest, in motion (Figure 39). Powder sample placed in a vessel (25 mm x 10 mL) underwent one conditioning cycle and was then compacted at 9 kPa. The vessel was split, and the shear cell test was then operated using a shear cell head. The powder was first re-compacted at 9 kPa to remove any disturbance caused by the split and to ensure that the sample surface was suitably consolidated. Then, a pre-shear was applied to achieve a steady state of the powder bed. After that, at decreasing normal stresses from 7 to 3 kPa by 1 kPa steps, the shear cell was rotated; and the incipient shear stress, which is the minimum shear stress required to make the powder bed flow was recorded for each applied normal stress. The major principal stress (σ_1) and the unconfined yield stress (σ_c) was determined by the FT4 software using the yield-locus approach and was permitted to calculate the flow factor (ff, equation 12).

$$ff = \sigma_1/\sigma_c \tag{12}$$

Powder flowability can be deduced from flow factor values by using Jenike’s classification:

- ff < 1: not flowing
- 1 < ff < 2: very cohesive
- 2 < ff < 4: cohesive
- 4 < ff < 10: easy flowing
- 10 < ff : free flowing



Shear cell above sample vessel

Shear cell test

Figure 39: Shear cell test using a FT4 powder rheometer (Freeman technology website).

3.4. Powder extract analysis

3.4.1. Extraction parameters

Aqueous extract. This extract was prepared in order to analyze the anthocyanin release in water. A 200 mL double-walled glass thermostatic beaker, connected to a circulating water bath, was used to maintain 50 mL distilled water at 20 or 50 °C. 50 °C is known to allow optimal extraction of *Hibiscus sabdariffa* anthocyanins (Chumsri et al., 2008). The temperature of 20 °C was chosen to investigate the possibility of performing cold extractions. Once the temperature was stable, 0.5 g powder was poured in water and the solution was stirred at 650 rpm for 30 min with a 1.5 cm magnetic stirrer. Extracts produced at 20 and 50 °C were sampled at 10, 20, 30 s, then every 30 s during 5 min, and finally after 30 min extraction for following analyses.

Methanol extract. Methanol extract was prepared by diluting 0.5 g of hibiscus powder in 10 mL methanol / water (70/30 v/v). The solution was stirred for 18 h using a magnetic stirrer at ambient temperature. This extract served to analyze the antioxidant activity of samples.

3.4.2. Anthocyanin content and antioxidant activity

3.4.2.1. Color of extracts, an indicator of anthocyanin presence

Two milliliters of aqueous extracts obtained at 50 °C were centrifuged (Minispin plus, Eppendorf AG, 22331 Hamburg, Germany) at 23 548 × g for 30 s. Red color intensity (CI, equation 13) and browning index (BI, equation 14) were evaluated by measuring the absorbance (A) of the supernatant of diluted centrifuged extracts (dilution factor of 1/10) at 520 nm (A_{520}) and 430 nm (A_{430}), respectively, using a UV/visible spectrophotometer (Shimadzu UV 1280, Kyoto, Japan). Indeed, the maximal light absorption of anthocyanins occurs at 520 nm wavelength, whereas molecules issued from browning have their absorbance peak at 430 nm (Cisse, 2010; Nguyen, 2018). Distilled water was used as a blank.

$$CI = A_{520} \tag{13}$$

$$BI = A_{430} / A_{520} \tag{14}$$

3.4.2.2. Anthocyanin content quantification

The differential pH method (**Figure 40**) was employed to measure the concentration of monomeric anthocyanins in extracts obtained at 20 and 50 °C. This method assumes that monomeric anthocyanins have little or no absorbance at pH 4.5 but high absorbance at pH 1.0 (Cisse, 2010; Lee et al., 2005). Buffers at pH 1 and 4.5 were prepared according to the method of Lee et al. (Lee et al., 2005). The pH 1 buffer solution (0.025 M, potassium chloride, KCl) was prepared by dissolving 1.86 g KCl in 980 mL distilled water. The pH was adjusted to 1.00 (± 0.05) with molar HCl. Preparation of the pH 4.5 buffer (0.4 M, sodium acetate, ($CH_3CO_2Na{\cdot}3H_2O$)) was prepared by dissolving 54.43 g $CH_3CO_2Na{\cdot}3H_2O$ in 960 mL distilled water. The pH was adjusted to 4.50 (± 0.05) with molar HCl.

One milliliter of extracts centrifuged at 23 548 × g for 30 s were diluted with 8 and 4 mL of pH 1 and 4.5 buffers, respectively. Between 20 and 50 min after dilution, the absorbance of the diluted extracts was read at 520 and 700 nm using a UV-visible spectrophotometer (Shimadzu UV 1280, Kyoto, Japan) with distilled water as blank. The measurement of the absorbance at 700 nm allowed considering the turbidity of the collected extracts. Measurements were also performed every 5 min until 30 min.

The absorbance of monomeric anthocyanins was calculated according to equation 15:

$$A = (A_{520\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{520\,nm} - A_{700\,nm})_{pH\,4.5} \qquad (15)$$

The concentration of anthocyanins was calculated by considering the molecular weight and extinction coefficient of delphinidin-3-xylosylglucoside, one of the major molecules of *Hibiscus sabdariffa*, according to equation 16:

$$[Anthocyanin] = (A \times MM \times F_d \times 10^3)/(\mathcal{E} \times l) \qquad (16)$$

With:

- [Anthocyanin]: concentration of monomeric anthocyanins expressed in delphinidin-3-xyloglucoside equivalents (mg.L^{-1}),
- MM: molar mass of delphinidin-3-xyloglucoside (577 g.mol $^{-1}$),
- F_d: dilution factor,
- Ɛ: molar extinction coefficient of delphinidin-3-xyloglucoside (26 000 L.mol^{-1}.cm^{-1}),
- l: length of the spectrophotometer cell (1 cm).

The anthocyanin release time was defined as the time for which the solution reaches 90 % of its maximum anthocyanin concentration.

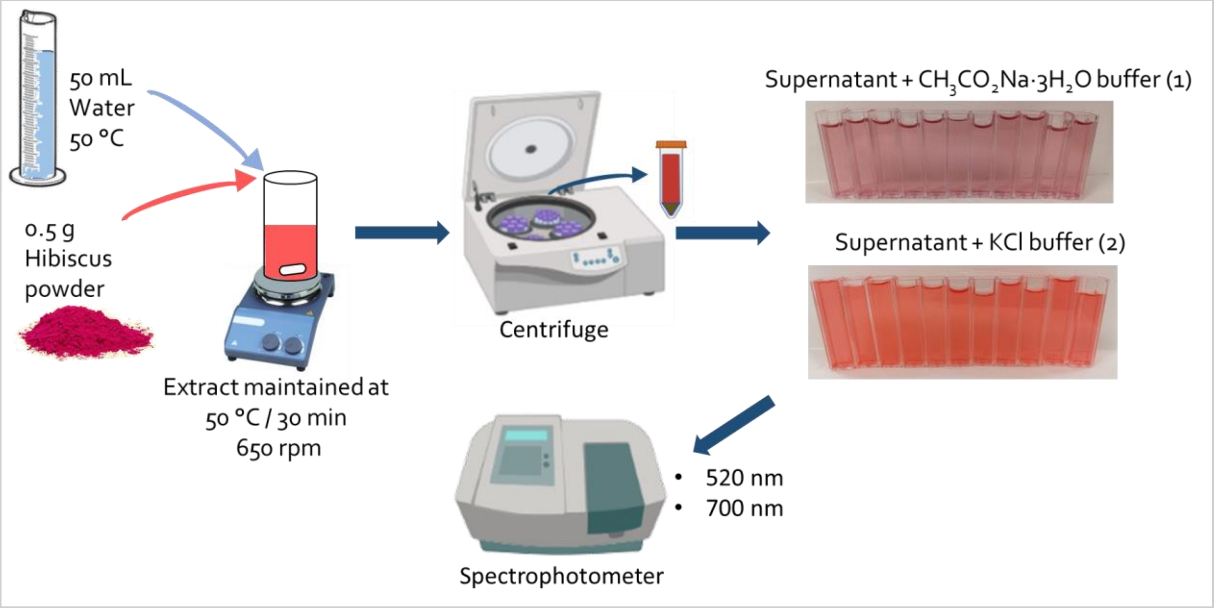


Figure 40: Anthocyanin concentration measurement by the pH differential method.

3.4.2.3. Identification of soluble molecules

Molecules found in hibiscus extracts were separated, detected, and identified using a ThermoVanquish™ quaternary Ultra high-performance liquid chromatography (UHPLC) system coupled with a photodiode array detector (PDA) and a ThermoScientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer equipped with an atmospheric pressure ionization interface operating in electrospray mode (ESI). Five microliters of hibiscus extracts (obtained at 50 °C) were injected on a Hichrom Alltima C18 column (150 mm × 2.1 mm; 5 µm silica beads) maintained at 25 °C. The flow rate was set at 200 µL.min^{-1} and mobile phases consisted in 0.1 % formic acid in water (solvent A) and 0.1 % acetonitrile and formic acid (solvent B). Elution was performed with a linear gradient from 5 % to 95 % B in 40 min. Mass analysis was carried out in ESI negative mode (spray voltage set at -2.5 kV) and in positive ion mode (spray voltage set at + 3.5 kV), with the same source parameters: sheath gas, auxiliary gas and sweep gas set (in arbitrary units.min^{-1}) at 35, 7 and 10, respectively; vaporizer and ion transfer tube temperatures both set at 300 °C. The powders were analyzed in the full-scan MS (150 – 2 000 *m/z)* and the HS_T2_B14 powder compounds extracts were monitored through *MS/MS. The structural identification was done in two ways: manually (publications) and automatically using databases proposed by the Compound Discoverer software.*

3.4.3. Antioxidant activity

Antioxidant activity was determined by two methods: PAOT Liquid Technology and ABTS method.

3.4.3.1. PAOT Liquid Technology

This method consists in recording the electrochemical potential changes of a reaction medium containing the powder sample using the PAOT Liquid Technology® apparatus (European Institute of Antioxidants, France). This technique consists of a measuring cell compartment and two microelectrodes, one for reference and the other for measurement. The reaction medium was composed of 1 mL physiological liquid at pH ranging from 6.7 to 7.2, and a free radical molecule considered as an oxidizing mediator. The electrodes were immersed in the reaction medium at a temperature of 25 ± 1 °C, and then 1 g of hibiscus powder was added. The oxidizing mediator oxidizes the antioxidants of hibiscus powder and this reaction results in a change in the electrochemical potential of the reaction medium linked with the evolution of concentrations of oxidized and reduced forms of the oxidizing mediator (Pincemail et al., 2019).

The antioxidant activity (AA), expressed as PAOT score per gram of product, was calculated according to equation 17 (Pincemail et al., 2019).

$$AA\ (\text{PAOT score/g product}) = \ \left(E_{product\ 10} - E_{product\ 0}\right) / \ E_{product\ 0} \ \times 100 \qquad (17)$$

With:

- $E_{product\ 10}$: electrochemical potential of the reaction medium 10 min after powder addition ($J.mol^{-1}$),
- $E_{product\ 0}$: the electrochemical potential of the reaction medium immediately after powder addition n ($J.mol^{-1}$).

Since this method is expensive, the evaluation of the activity of the powders from the second harvest was done using conventional analytical methods such as the ABTS method.

3.4.3.2. ABTS Method

Antioxidant activity was assessed by the ABTS (2,2'-azino-bis 3-ethylbenzothiazoline -6-sulfonic acid) method, which determines the free radical scavenging capacity of a sample. The ABTS cation radical was generated by mixing exactly 39.2 mg of 98 % ABTS (7 mM) and 6.62 mg of potassium persulfate (2.45 mM), each of them previously dissolved in distilled water in a 10 mL volumetric amber flask. The mixture reacted in the dark for 16 h at room temperature. The absorbance of the ABTS radical cation solution was adjusted with methanol to 0.70 ± 0.02 units at a wavelength of 734 nm (Deli et al., 2019a).

The 10 mM gallic acid solution (the standard) was prepared by weighing 25.8 mg (97.8 % purity) into 10 mL amber vial, which was then dissolved with methanol / water (70/30 v/v). From the gallic acid solution, dilutions were made with methanol / water (70/30), to obtain concentrations of 0.125, 0.25, 0.50, 1.0, 1.5, 2.0 mM which were the different points of the standard curve.

In a dark environment, 40 µL aliquot of each diluted gallic acid solution was transferred to 10 mL amber vials. Then 4 mL of the radical ABTS solution was added and vortexed. The absorbance (at 734 nm) was then read after exactly 30 min with a spectrophotometer (Shimadzu UV 1280, Kyoto, Japan). Pure methanol was used for the blank.

One milliliter of methanol extract was transferred to a 20 mL vial, and diluted with 70/30 methanol (v) / water (v). Then, 40 µl of the diluted powder extract was introduced into a 10 mL amber vial, and 4 mL of the ABTS solution was added. The mixture was vortexed and kept in the dark for 30 min. The absorbance was then measured at 734 nm.

The antioxidant activity equivalence of powder was found from the interpolation of the calibration curve in mM of gallic acid which is multiplied by the dilution factor (F_d = 20/1), by the solvent volume (L) and by 10^3, all divided by the weight of the sample in grams. It is thus expressed in µmol gallic acid per gram of sample (equation 18).

$$\text{µmol Gallic acid/g powder} = \frac{(mM\ of\ Gallic\ acid * F_d * extract\ sample * 10^3)}{powder\ mass\ (g)} \tag{18}$$

Using this method, the antioxidant activity measurement was performed when producing the powder and after 30 days of storage at 4 °C.

3.5. Powder reconstitution

3.5.1. Particle size and shape evolution

The dynamic image analysis system QICPIC (Sympatec GmbH, Clausthal-Zellerfeld, Germany) was used in wet dispersion mode using the SUCELL system in order to monitor the particle size and shape evolution during reconstitution assays (Figure 41). To this end, 0.16 g of hibiscus powder was poured into 620 mL distilled water in the stainless-steel tank of the QICPIC at ambient temperature (22 ± 2 °C).

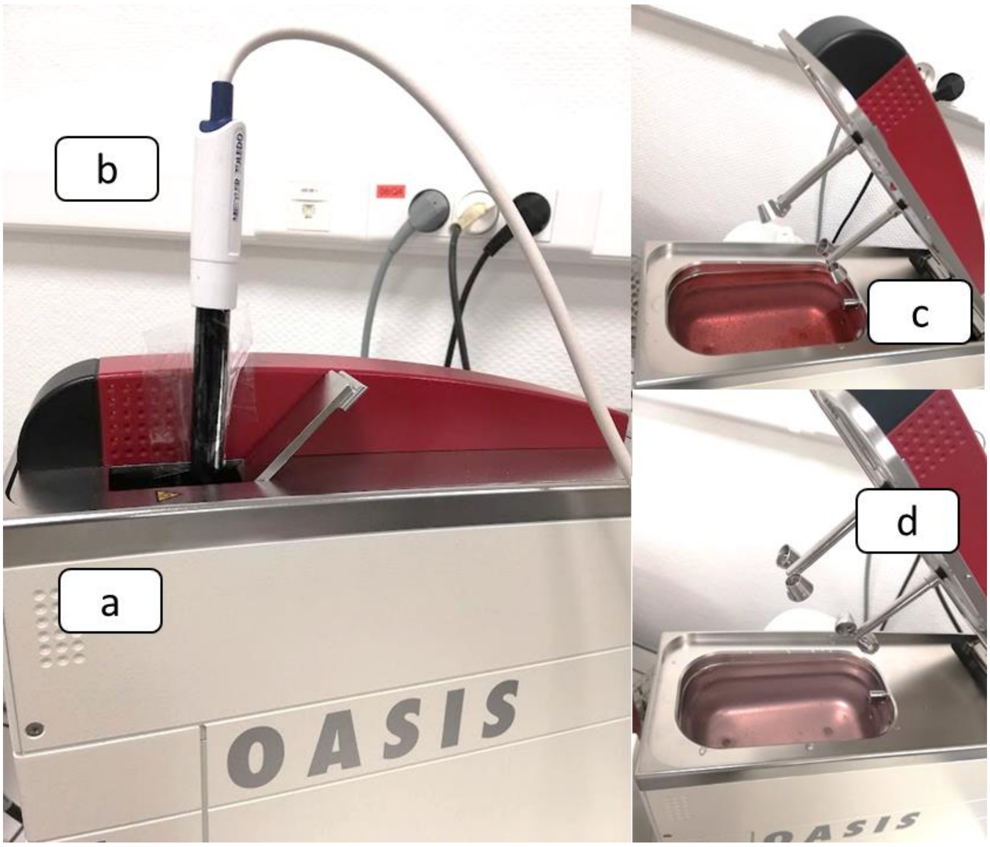


Figure 41: Experimental setup for the evaluation of particle size and shape evolution during reconstitution of hibiscus powders in water, a - SUCELL/L wet dispersion unit, b - conductivity probe, c - tank, d- immersible agitator (viscojet).

The measurement was then started 20 s after powder addition. This duration corresponds to the time required to fill the measurement cell and check optical concentration, which must be comprised between 0.1 and 2.0 %. The solution was homogenized at 650 rpm using two immersible QICPIC stirrers (Viscojet, Germany). A peristaltic pump (set at 100 %) ensured a constant flow rate in the measuring cell. The M4 optical module (1.1 – 2 253 µm) and a 0.5 mm cuvette were used for fine powders, and the M7 optical module (4.2 – 8 665 µm) and a 2 mm cuvette for coarse powders. Measurements were performed every 30 s for 30 min.

The average particle size and the number of particles in the solution during powder reconstitution were followed. Normalization of the results required first evaluating the difference between the value to be normalized and the minimum value of the same data set. Then, this difference was related to the difference between the maximum value and the minimum value (equation 19). The particle diameter at dry state was taken as particle size at zero time (Figure 42).

$$y_N = \frac{y - y_{min}}{y_{max} - y_{min}} \qquad (19)$$

Where:

- y_N: The normalized value,
- y: The value to be normalized,
- y_{min}: The minimum value of the same data set than y,
- y_{max}: The maximum value of the same data set than y.

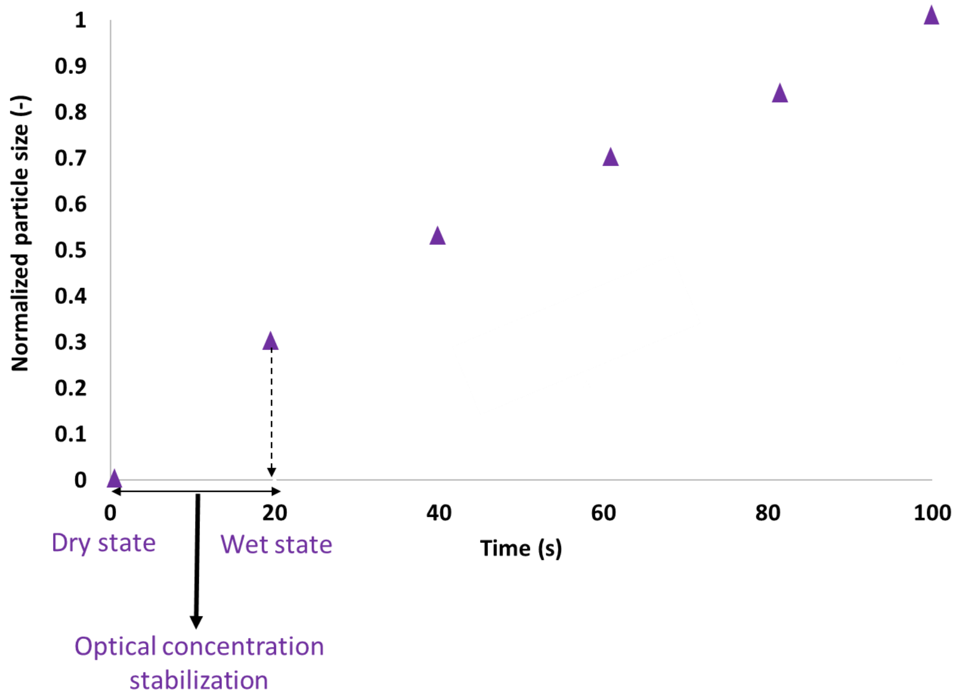


Figure 42: A representation of the particle size evolution during reconstitution.

The curve representing the temporal evolution of normalized particle number had two asymptotic trends (Figure 43). The time at which intersected the tangents to the curve in the two phases was taken as the transition time between two identified dispersion phases. The swelling (resp., dispersion) rate was calculated as the slope of the linear trend curve of the normalized average particle size (resp., particle number) during the increasing phase.

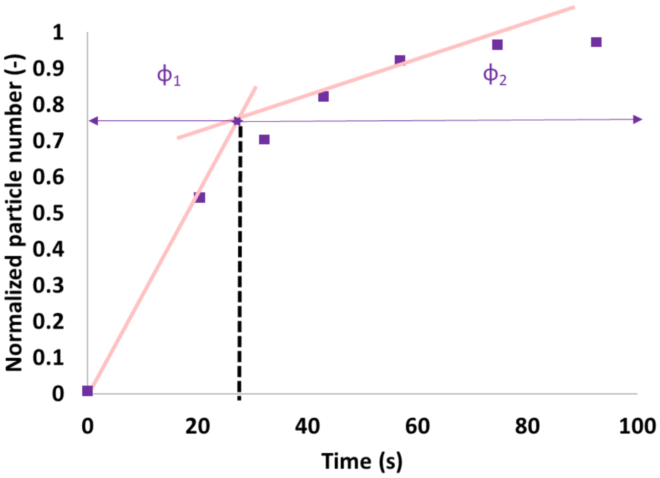


Figure 43: A representation of the particle number evolution during reconstitution with phase 1 (Φ_1) and phase 2 (Φ_2).

3.5.2. Conductimetry

During powder reconstitution, conductivity of the solution was measured using a Mettler Toledo GmbH conductimeter (SevenCompact S230, Greifensee, Switzerland) with the probe (Mettler Toledo, InLab 731 ISM, Switzerland) inserted vertically in the QICPIC stainless-steel tank. Calibration was performed at 25 °C with a liquid of 1 413 µS.cm^{-1} conductivity. The reconstitution time was defined as the time for which the solution reaches 90 % of its maximal conductivity (Gaudel et al., 2022; Mitchell et al., 2015).

3.5.3. Water-insoluble material

The insoluble matter (IM) of extracts obtained at 20 and 50 °C was quantified using a method adapted from Cisse (Cisse, 2010). Fifty milliliters of extracts were centrifuged (Heraeus, ThermoFisher Scientific, Germany) at 2 268 × g for 10 min. The supernatant was removed and replaced by 35 mL distilled water at 50 °C. The solutions were stirred with a vortex at 2 000 rpm for 10 s and then centrifuged using the same conditions as before. The supernatant was removed and the pellet was collected in a dish to be dried at 103 °C for 24 h. The supernatant residual dry matter was finally weighed and the proportion of insoluble material (IM, expressed in %) was calculated according to equation 20.

$$\mathrm{IM} = \frac{M_{residual}}{M_{powder}} \times 100 \qquad (20)$$

With:

- $M_{residual}$ (g): mass of water-insoluble material,
- M_{powder} (g): mass of the powder dry matter added in the solution.

3.5.4. Brix and pH

The pH of reconstituted powders was measured using a pH-meter (Mettler toledo, Five easy plus). Brix of solutions (90 % of water and 10 % powder) was measured at 20 °C using a hand-held refractometer (ATAGO, Master-M).

3.6. Statistical analysis

The statistical analyses were performed using XLSTAT 2019.4.2 (ADDINSOFT, France) add-on for Microsoft Excel software. The experiments were performed in triplicate. Reported values are means ± standard deviations. Data were subjected to a one-way analysis of variance (ANOVA) to determine if there were significant differences between means ($p \leq 0.05$). The Tukey's honestly significant difference (HSD) test was used to sort powders into groups significantly different between them. A non-parametric test, precisely the Kruskal-Wallis test was applied when the assumptions of ANOVA were not met. The Pearson correlation coefficient was also evaluated to measure the degree of linear correlation between two variables.

In addition, a Principal Component Analysis (PCA) was running to identify the predominant properties that characterize hibiscus powders.

Chapitre 4 : Results and discussions

Introduction à la première partie des résultats

A l'issue de l'état de l'art, il apparait que la production de poudres par le couplage du séchage et du broyage est pertinente puisqu'il permet l'obtention d'un produit très peu transformé et, si les opérations unitaires sont correctement menées, permet de préserver les propriétés originelles des calices d'hibiscus.

La conversion de l'hibiscus sous forme de poudre permet de garantir la disponibilité, l'accessibilité aux produits (périssables et saisonniers en particulier) quel que soit le lieu ou la période de l'année. Les produits pulvérulents présentent aussi un avantage au moment de l'extraction des biomolécules car la plus grande surface spécifique permet un contact plus important avec l'eau. Enfin, ce sont des produits facilement manipulables (à condition que la granulométrie ne soit pas trop fine 60 – 100 µm) que ce soit par des utilisateurs intermédiaires (préparation de formulations par des industriels) ou le consommateur final. La production des poudres par le couplage du séchage et du broyage fait l'objet de cette première partie des résultats (Figure 44). A l'issue de chaque production, l'impact des opérations unitaires sur les propriétés physicochimiques et l'aptitude à l'écoulement des poudres ont été évalués. Les calices ont été séchés au soleil et/ou à l'étuve pendant 1 ou 2 h puis broyés à 10 000, 12 000 ou 14 000 tr.min^{-1}. L'augmentation de la durée de séchage et/ou de la vitesse de broyage, facilite la réduction des calices en de fines poudres polydisperses à particules irrégulières, et contenant une faible proportion de particules fibreuses. Ces propriétés physicochimiques ne sont pas sans impact sur les propriétés d'écoulement : les poudres deviennent moins cohésives ; elles sont donc moins sensibles à la compression et au test de cisaillement, et par conséquent elles s'écoulent bien.

L'intensité de la couleur rouge des solutions préparées lors de la reconstitution des poudres est liée à la teneur en anthocyanes. Cette couleur est plus intense lorsque la durée du séchage est courte et que la vitesse de broyage est élevée. Cependant, pour réduire le taux de matière insoluble des poudres, une durée de séchage et une fréquence de broyage élevées seraient nécessaires. Le choix de la combinaison de la durée de séchage et de la fréquence de broyage est crucial pour obtenir une poudre qui s'écoule facilement, et présente simultanément une longue durée de conservation et une coloration rouge suffisamment attrayante pour les consommateurs. Cette première partie des résultats a fait l'objet d'une publication scientifique publiée dans Particulate Science and Technology :

C.U. M'BE, J. SCHER, J. PETIT, N.G. AMANI, J. BURGAIN, Relationship Between Drying and Grinding Parameters and Physicochemical Properties of *Hibiscus sabdariffa* Calyx Powders, *Particulate Science and Technology*, Taylor & Francis, 2022, pp. 1–10, doi:10.1080/02726351.2022.2032508.

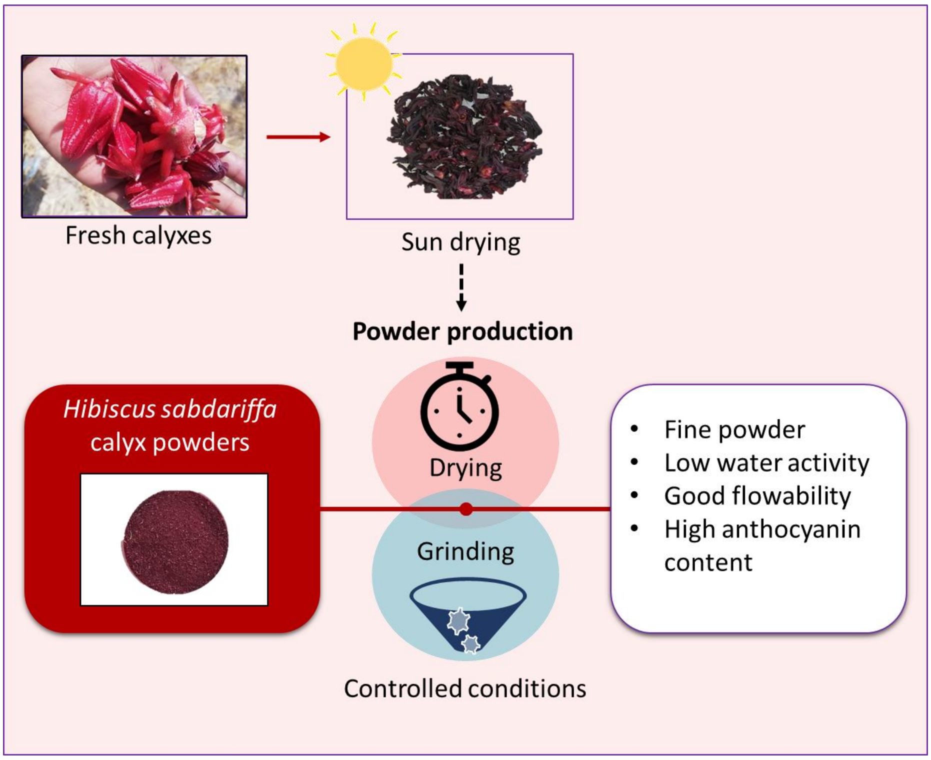


Figure 44: Résumé graphique de la production des poudres d'*Hibiscus sabdariffa* obtenues par séchage solaire (suivi d'un étuvage) et broyage.

4.1. Relationship between drying and grinding parameters and physicochemical properties of *Hibiscus sabdariffa* calyx powders: Article n°1

4.1.1. Introduction

Hibiscus sabdariffa is an annual herbaceous plant of the Malvaceae family cultivated in many tropical and subtropical regions (Ahmed et al., 2019; Chumsri et al., 2008; Cisse, 2010; Da-Costa-Rocha et al., 2014; Ramírez-Rodrigues et al., 2011; Sinela, 2016). The leaves and calyxes of this plant are all edible, and stems are employed for cattle feeding or textile. There are two types of calyxes of *Hibiscus sabdariffa* – red and white ones – with a similar chemical composition (Ahmed et al., 2019). The red calyxes are particularly prized in the producer countries and also in foreign countries such as the United States of America and Germany (M. J. P. Monteiro et al., 2019; Plotto et al., 2004b). The red color is the first appealing criteria of calyxes of *Hibiscus sabdariffa* followed by its sour-fruity taste and its high content in antioxidants. The *Hibiscus sabdariffa* calyxes are a rich source of water (90 % water content), proteins, fibers, and carbohydrates (Cisse, 2010). These calyxes are also particularly rich in mineral elements (K, Ca, Mg, Fe, Mn, Zn), organic acids (succinic, oxalic, malic, citric, stearic, tartaric, and ascorbic acids), and anthocyanins (Ahmed et al., 2019; Cisse, 2010; Da-Costa-Rocha et al., 2014). Anthocyanins are responsible for the red color of *Hibiscus sabdariffa* calyxes and play an important role as an antioxidant.

This calyx composition, especially its high anthocyanin content, makes the *Hibiscus sabdariffa* a very coveted plant part in several fields of activity such as cosmetics, pharmacy, medicine, and the food industry. In medicine*, Hibiscus sabdariffa* calyxes are used for their diuretic, febrifugal, antihelminthic, antimicrobial, hypotensive, and hypocholesterolemic activities and for its ability to stimulate intestinal peristalsis (Da-Costa-Rocha et al., 2014; Fullerton et al., 2011; Johansen et al., 2005; Lee et al., 2009; Paim et al., 2017; Peng et al., 2011; Pérez-Torres et al., 2013; Seck et al., 2018). Also, *Hibiscus sabdariffa* calyxes are used in the food industry mainly to produce fresh beverages (fermented drinks, wine, etc.) and hot beverages like infusions. Cocktails, syrups, fruit salads, and pastry foods are also prepared from *Hibiscus sabdariffa* calyxes. In addition, delicious jellies, jams, and ice creams are manufactured and most appreciated by many populations all over the world (Cisse, 2010; Plotto et al., 2004b). For all these applications, hibiscus calyxes are either employed in fresh form or stabilized to facilitate transport and ensure good quality upon reception. The basic stabilization process consists of solar-drying, which allows the reduction of moisture content and prevention of microbial contamination (Labuza, 1977). As a result, it extends the shelf-life and controls the seasonality issue. Aqueous extracts of *Hibiscus sabdariffa* calyxes may also be prepared before being freeze or spray-dried. Spray-drying is the most applied process to obtain powders (Djaeni et al., 2018). However, the quality of *Hibiscus sabdariffa* calyx powders is altered during the stabilization process because of the degradation of anthocyanins. High extraction temperature and/or duration lead to higher extraction yields of dry matter but also favor the loss of anthocyanins by thermal alteration. The thermal degradation of anthocyanins occurs by the rupture of these biomolecules, leading to the change in color of final products and a reduction of the antioxidant activity (Chumsri et al., 2008; Cisse, 2010; Eroğlu et al., 2018; Idham et al., 2012; Osman and Endut, 2009a; Tsai et al., 2002).

To propose another powdering method, the current work focuses on the assessment of the influence of drying, which is achieved by sun-drying followed by oven drying, coupled with grinding on the functional properties (flowability, preservability) of *Hibiscus sabdariffa* calyx powders. To better understand the link between processing conditions and powder functionalities, their physicochemical properties (water content, water activity, particle size and shape, red color intensity, insolubility) were also determined.

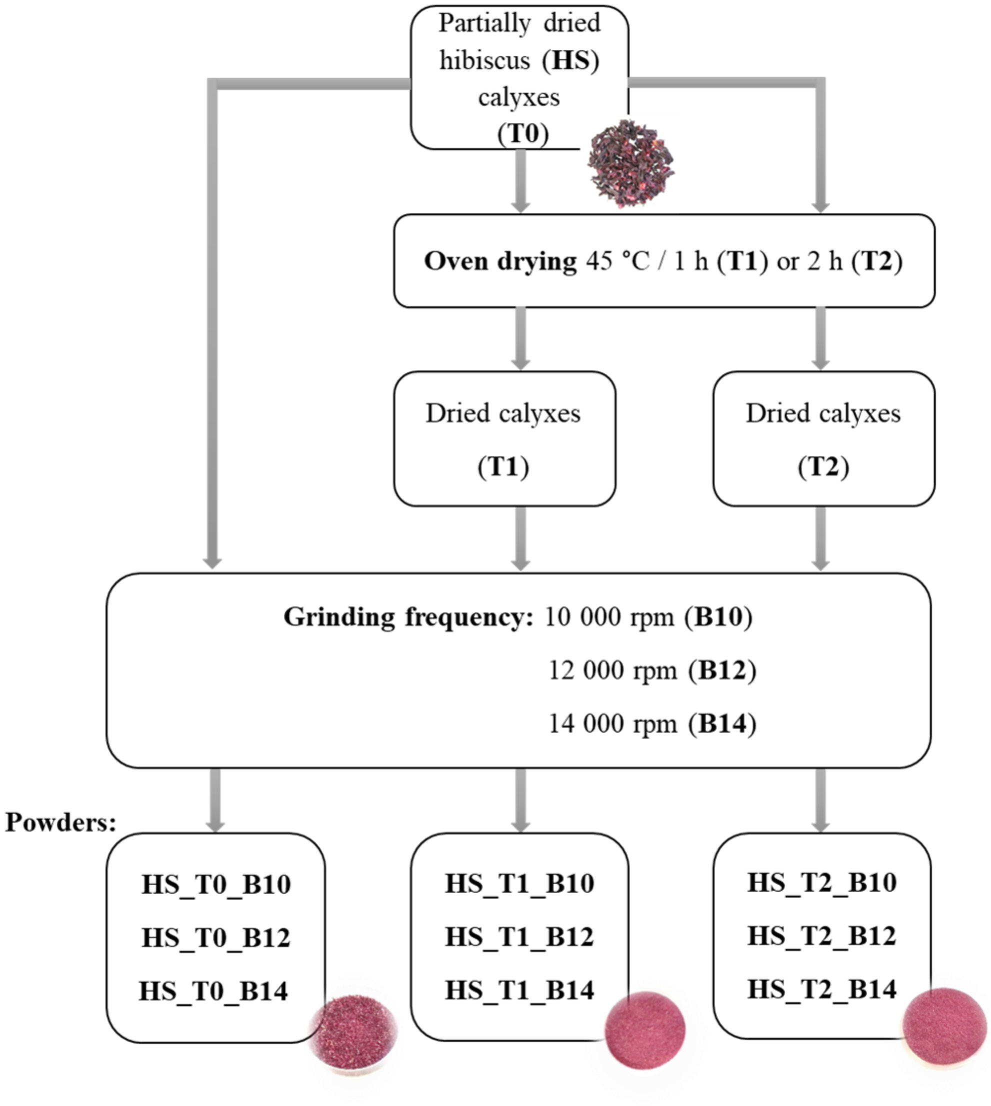
4.1.2. Material and methods

Plant material (see section 3.1.1– First calyx harvest)

Calyxes from the first harvest were subjected to solar-drying with additional oven drying (1 h and 2 h / 45 °C).

Powder production (see section 3.2.1)

The production process is reminded in the figure below.



Manufacturing process of pre-treated *Hibiscus sabdariffa* calyx powders (Figure 31 , section 3.2.1).

Powder physicochemical characterization (see section 3.3)

- Water content and water activity
- Color measurements
- Particle size distribution
- Particle morphology
- Powder flowability

Powder extract analysis (see section 3.4)

- Powder reconstitution parameters
- Anthocyanins content determination
- Water-insoluble material

Statistical analysis (see section 3.6)

4.1.3. Results and discussion

4.1.3.1. Impact of processing parameters on powder properties

4.1.3.1.1 Water content

Sun-dried *Hibiscus sabdariffa* calyxes (18.50 g / 100 g water content on wet basis), lost 18 % of their initial water content after 1 h oven drying (T1) to reach 15.14 ± 0.40 g / 100 g (Table 3). After 2 h oven drying (T2), the water content of calyxes was 14.24 ± 0.59 g / 100 g, which corresponds to a 6 % further reduction of initial water content compared to T1. Water activity was lowered by 16 % ($a_w = 0.56 \pm 0.02$) compared to T0 after the first hour of oven drying (T1), then the calyxes reached 0.53 ± 0.01 water activity after 2 h oven drying (T2). Beyond 1 h drying time, the water content of the calyxes did not reduce by much because of the hardness of the calyx surfaces and the removal of the quasi totality of free water. The remaining water content was likely bound water. The T1 and T2 oven-dried calyxes were free of microbial growth ($a_w < 0.65$) but remained sensitive to enzymatic and non-enzymatic reactions and lipid oxidation (Labuza, 1977). Sun-dried calyxes had the highest water activity of 0.65 ± 0.01 and were thus more prone to alteration by bacteria and fungi (Adebayo-tayo, B. C. and Samuel, U. A., 2009), lipid oxidation, and enzymatic reactions.

The grinding step systematically led to a reduction of water content and water activity (Table 3). This reduction was greater for T0 calyxes: T0 powders ground at 10 000 rpm (T0_B10) had a 16 % lower water content than the unground T0 calyxes. Water content decreased by 2 % when increasing the grinding frequency from 10 000 (B10) to 12 000 rpm (B12) and by 6 % when the grinding frequency was raised from 12 000 (B12) to 14 000 rpm (B14).

To obtain powders, grinding T1 and T2 calyxes at B10 frequency resulted in a 7 % decrease in water content. With higher grinding frequencies, the water content remained unchanged ($p > 0.05$). The powdered T0 calyxes also had a reduced water content when ground at B10. This water content was further reduced ($p < 0.05$) when increasing the grinding frequency from B10 to B14.

During grinding, the friction of the product between the rotor and the mill screen promotes local heating (Karam et al., 2016). Therefore, it was expected that friction would increase with grinding frequency. However, the grinding temperature only reached 30 °C in all experiments and did not vary with grinding frequency. Therefore, this temperature was not sufficient enough to be responsible for significant evaporation or a reduction in water content. On another note, grinding results in an increase in the specific surface area of the particles which would have favored the transfer of water to the ambient air, which could justify this reduction in water.

Table 3: Physicochemical properties of *Hibiscus sabdariffa* calyx powders.

Sample	Sample acronym	Water content (g / 100 g on wet basis)	Water activity (-)	Span (-)	Cohesion (kPa)	Flow factor (-)	Fiber (%) (on wet basis)
Calyxes	Dried calyxes (T0)	18.50 ± 0.36 ^{a}	0.65 ± 0.01 ^{a}	-	-	-	-
	Dried calyxes (T1)	15.14 ± 0.40 ^{b}	0.56 ± 0.02 ^{bc}	-	-	-	-
	Dried calyxes (T2)	14.24 ± 0.59 ^{de}	0.53 ± 0.01 ^{bc}	-	-	-	-
Powders	HS_T0_B10	15.49 ± 0.22 ^{bc}	0.55 ± 0.02 ^{bc}	2.11 ± 0.06 ^{e}	1.77 ± 0.05 ^{ab}	2.89 ± 0.1 ^{e}	1.45 ± 0.2 ^{a}
	HS_T1_B10	14.15 ± 0.03 ^{de}	0.59 ± 0.01 ^{c}	2.24 ± 0.06 ^{de}	1.62 ± 0.24 ^{abc}	3.28 ± 0.36 ^{cde}	0.56 ± 0.01 ^{b}
	HS_T2_B10	13.31 ± 0.02 ^{f}	0.45 ± 0.02 ^{d}	2.42 ± 0.1 ^{bcd}	1.2 ± 0.11 ^{bcd}	4.52 ± 0.32 ^{bc}	0.42 ± 0.03 ^{bcd}
	HS_T0_B12	15.80 ± 0.14 ^{b}	0.56 ± 0.01 ^{bc}	2.22 ± 0.05 ^{de}	1.11 ± 0.01 ^{cd}	4.39 ± 0.12 ^{bcd}	1.32 ± 0.14 ^{a}
	HS_T1_B12	14.19 ± 0.07 ^{de}	0.53 ± 0.00 ^{c}	2.56 ± 0.05 ^{abc}	1.11 ± 0.05 ^{cd}	4.56 ± 0.35 ^{bc}	0.53 ± 0.07 ^{bc}
	HS_T2_B12	13.29 ± 0.08 ^{f}	0.46 ± 0.02 ^{d}	2.56 ± 0.16 ^{abc}	0.82 ± 0.13 ^{d}	6.15 ± 0.97 ^{a}	0.26 ± 0.02 ^{d}
	HS_T0_B14	14.82 ± 0.12 ^{cd}	0.55 ± 0.02 ^{b}	2.31 ± 0.07 ^{cde}	1.98 ± 0.55 ^{a}	2.92 ± 0.66 ^{de}	1.21 ± 0.09 ^{a}
	HS_T1_B14	14.07 ± 0.05 ^{e}	0.53 ± 0.02 ^{c}	2.61 ± 0.06 ^{a}	1.00 ± 0.13 ^{cd}	4.97 ± 0.64 ^{ab}	0.28 ± 0.03 ^{cd}
	HS_T2_B14	13.14 ± 0.15 ^{f}	0.48 ± 0.01 ^{d}	2.80 ± 0.13 ^{a}	0.92 ± 0.10 ^{d}	5.5 ± 0.56 ^{ab}	0.27 ± 0.02 ^{cd}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p < 0.05$).

4.1.3.1.2 Particle size distribution

When increasing the drying duration, the particle sizes decrease whatever the grinding frequency (Figure 45). Significant size reductions were observed for all T1 powders compared to the reference powder (T0): 44 %, 50 %, and 52 % for B10, B12, and B14, respectively. The particle size of all T2 powders did not significantly differ from T1 powders. Differences of 14 %, 7 %, and 5 % between T1 and T2 powders were obtained for B10, B12, and B14, respectively. Therefore, 1 h oven drying had the most influence on grinding-induced particle size reduction. T0 (resp., T2) calyxes led to the largest (resp., smallest) average particle sizes in agreement with the Pearson coefficient, which shows a strong influence (R = - 0.83) of drying duration on particle size. Upon drying-induced water removal, the material collapses and becomes tougher, more brittle, or more friable. All of this facilitates its grinding. Consequently, the powder with the smallest water content resulted in the finest powder as highlighted by Sui et al. (2019). This observation is confirmed by Deli et al. (2019), who reported that sun-dried *Hibiscus sabdariffa* calyxes ground at 12 000 rpm had a average particle size of 125.65 ± 2.08 µm and water content of 7.83 ± 0.5 g / 100 g. Powders produced by Deli et al. (2019) – with the same equipment and in the same conditions as HS_T0_B12 powders of the present study – were finer, probably owing to their water content which was half of the hibiscus calyxes of the present study.

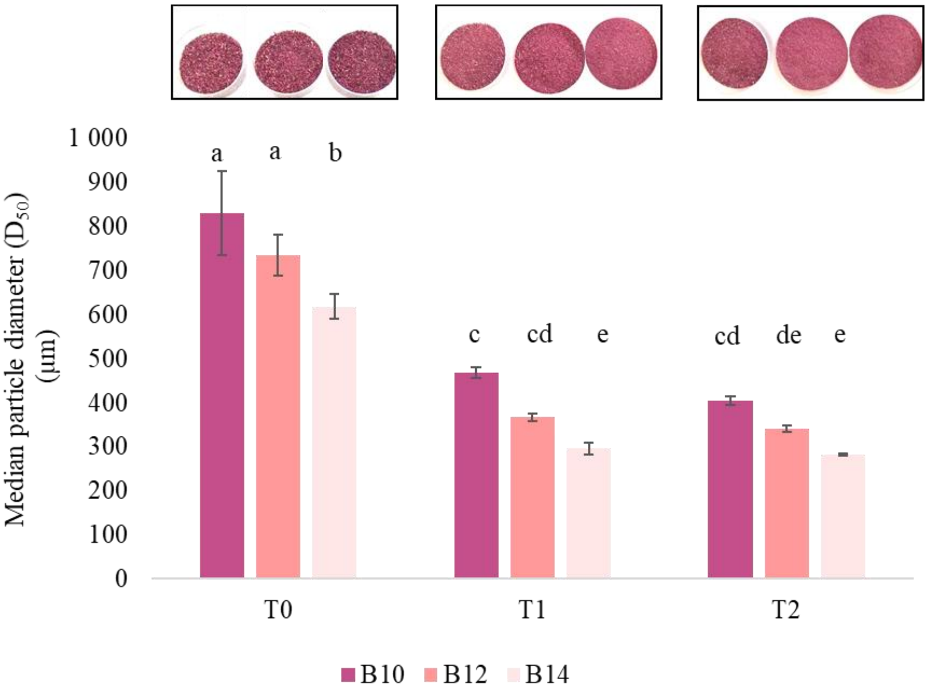


Figure 45: Average particle diameter of *Hibiscus sabdariffa* calyx powders (bars topped by different letters are significantly different according to Tukey's HSD test (n = 3, $p < 0.05$)).

As expected, the average particles size decreased at a higher grinding frequency (Figure 45). For T0, T1, and T2 powders, the average particle size was reduced by 11 %, 22 %, and 16 % respectively, from B10 to B12. From B12 to B14, the average particle decreased by 16 %, 20 %, and 17 % for T0, T1, and T2 powders respectively. The *Hibiscus sabdariffa* calyx particles became finer at higher grinding frequency, which is consistent with okra seed powders studied by Waiss et al. (2020). Increasing the grinding frequency confers more kinetic energy to the material to be ground, favoring its break upon impact on the tooth-shaped rotor and the crushing between the rotor and the ring sieve. Moreover, using a higher grinding frequency at constant grinding duration increases the total number of rotations of the rotor, leading to finer products. The finest powder was obtained from calyxes that were oven-dried for 2 h and ground at 14 000 rpm (HS_T2_B14). As previously mentioned, drying may increase calyx brittleness, thus favoring the grinding. All distributions were bimodal with span values ranging from 2.1 to 2.8 (Table 3), which points to the heterogeneity of *Hibiscus sabdariffa* calyx powders composed of two main populations: fine and large particles (Figure 46). Gnagne et al. (2017), Deli et al. (2019), and Waiss et al. (2020) showed that particle size distributions of powders issued from plant grinding were generally constituted of more than one population. For B12 and B14 powders, span values increased from T0 to T1 and did not significantly change with further drying duration. Grinding frequency also favored an increase in span for T1 and T2 powders, therefore the heterogeneity. The greater the grinding frequency, the finer the particles; and the greater the span, the more heterogeneous the powders.

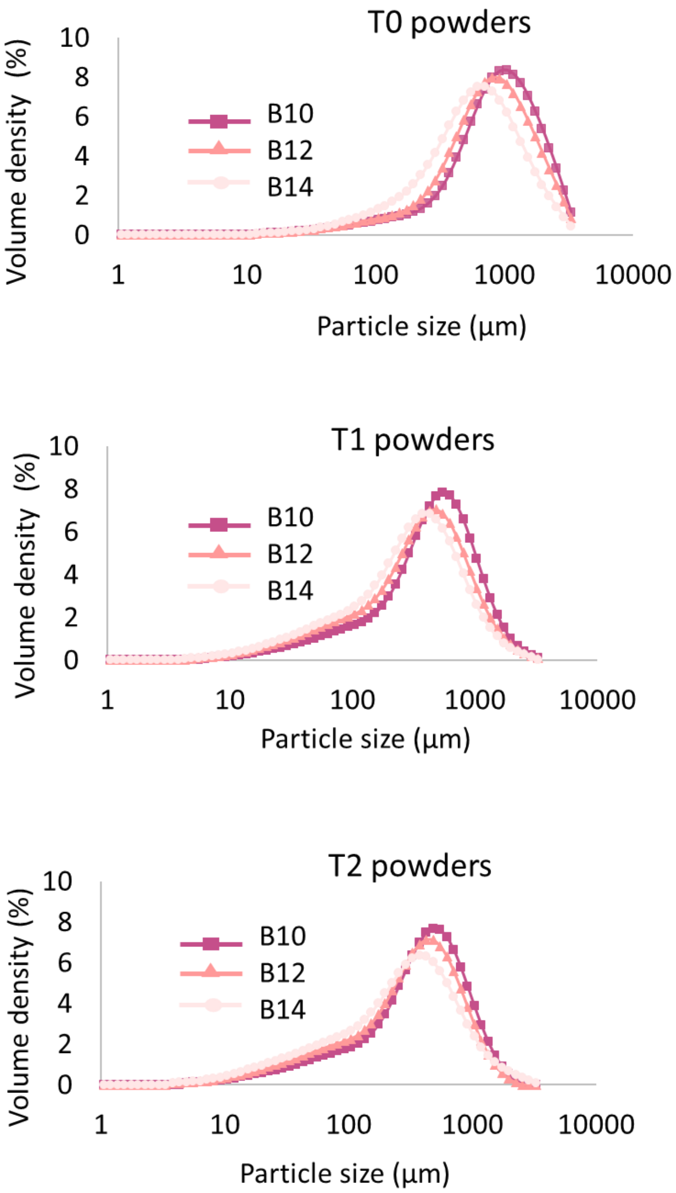


Figure 46: Particle size distributions of *Hibiscus sabdariffa* powders.

4.1.3.1.3 Powder color

Drying duration affects powder color saturation, as reflected by chroma results (Figure 47). It was generally observed that chroma value increased with drying duration. An observation confirmed by the Pearson coefficient (R = 0.58) indicates a moderately positive influence of drying duration on chroma. Indeed, the longer the drying time, the more intense the powder color. Chroma did not change after 1 h oven drying for B10 powders but increased after 2 h. The chroma significantly ($p < 0.05$) increased from T0 to T1 for B12 powders, and became almost constant from T1 to T2. These results show that drying duration led to the discoloration of hibiscus calyx powders, caused by the formation of drying-induced oxidized products. Chroma value was also affected by the grinding frequency, as suspected, but only for T1 powders (**Figure 47**). Chroma of these powders increased by 8 % from B10 to B12 and did not significantly change from B12 to B14. Higher grinding frequency favored more intense powders color, which is a result of the grinding-induced increase in particle specific surface area.

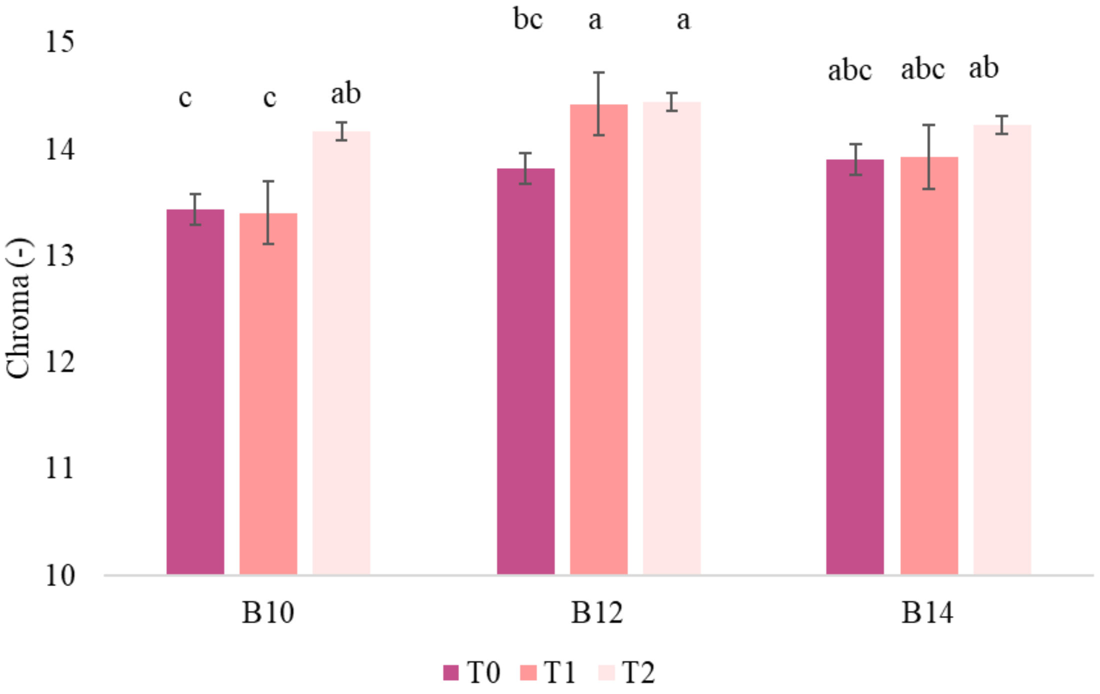


Figure 47: Impact of drying duration and grinding frequency on chroma of *Hibiscus sabdariffa* powders (bars topped by different letters are significantly different according to Tukey's HSD test (n = 3, $p < 0.05$)).

4.1.3.1.4 Powder flowability

Compressed bulk densities of T0 powders were significantly lower than those of T1 and T2 powders, depending on the grinding frequency (**Figure 48**). This result was expected since T0 powders had higher interparticular porosity (related to their irregular and large particles) than T1 and T2 powders (**Figure 49**).

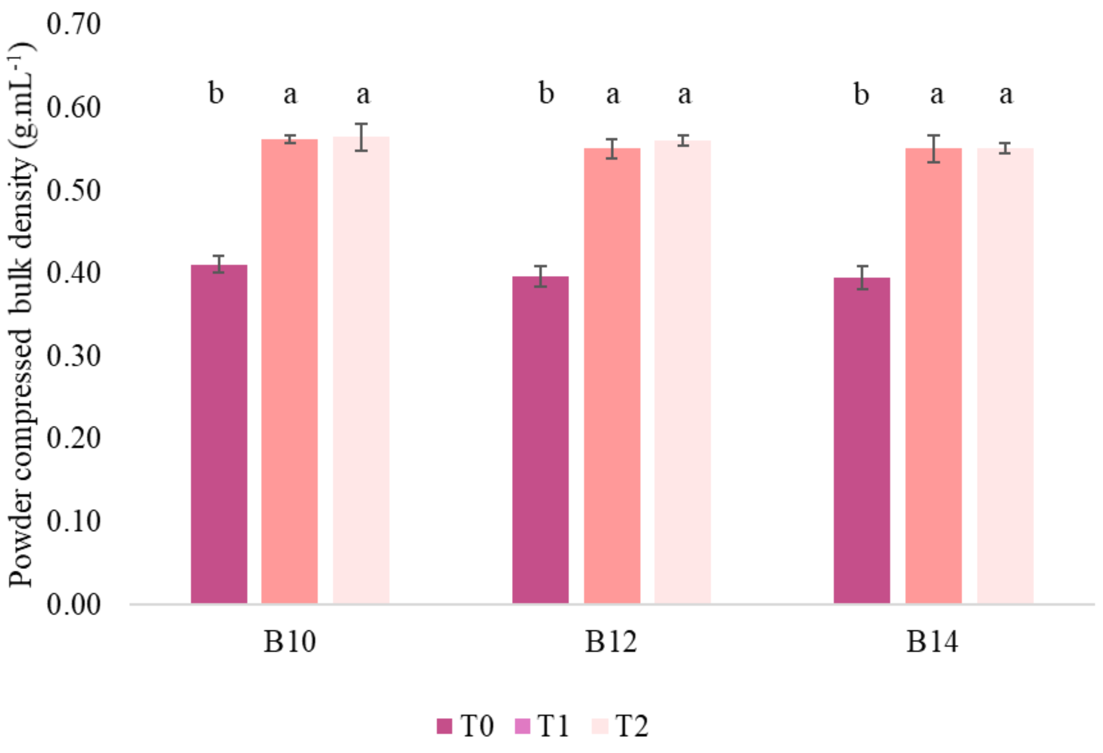


Figure 48: Impact of drying duration and grinding speed on compressed bulk density of *Hibiscus sabdariffa* calyx powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

T1 and T2 powders had similar bulk densities ranging from 0.55 ± 0.011 to 0.56 ± 0.014 g.mL^{-1}, which could be attributed to the similarity of their particle size distributions. Moreover, grinding frequency seemed to not have a significant influence on powder bulk density. The compressibility results are depicted in **Figure 50**-a. T1 and T2 powders had similar relatively low compressibility, whereas T0 powders were more compressible. The compressibility of T1 and T2 powders at 15 kPa normal stress was 12 % for B10 and B12 powders and 15 % for B14 powders. This low compressibility of T1 and T2 powders may be an indication of their better flowability than T0 powders (Gnagne et al., 2017). The close compressibilities of T1 and T2 powders were likely to result from their similar particle size distributions. Compressibility of T0 powders was more important: 21 %, 24 %, and 23 % for B10, B12, and B14 batches, respectively. The higher water content of T0 powders may contribute to their higher compressibility. Therefore, water has a plasticizing effect on solid materials, which increases their deformability upon compression and increases the stickiness of particles and their cohesion. This in turn enhances powder compressibility (Fitzpatrick et al., 2007; Zafar et al., 2017). Powder compressibility was also affected by grinding frequency. For T1 and T2 powders, compressibility was similar for B10 and B12 batches and increased for the B14 batch. This may be explained by the finest particles (i.e. lower average particle size) and the higher heterogeneity (i.e. higher span) of T1_B14 and T2_B14 powders. Indeed, more heterogeneous powders generally have higher compressibility (Petit et al., 2017). Since compressibility is representative of the intergranular forces and therefore, indirectly of the cohesion of powders (Petit et al., 2017), it is important to carefully handle compressible powders to avoid caking during handling, packaging, or storage.

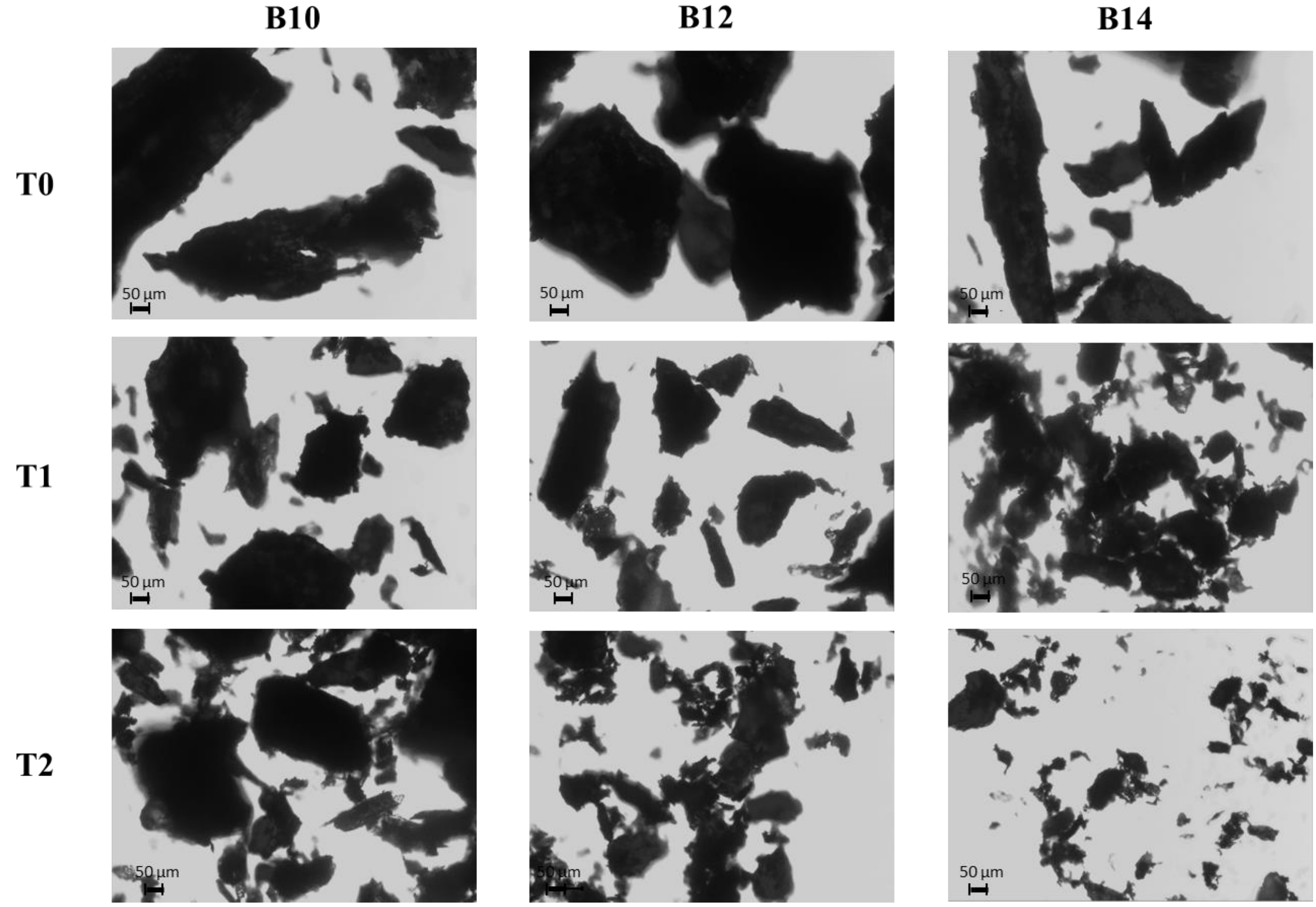


Figure 49: Morphological observation of particle surface of *Hibiscus sabdariffa* powders.

The evolution of incipient shear stress when changing the applied normal stress is presented in Figure 50-b. The application of normal stress to the powder bed causes particle deformations leading to a decrease in the interparticle distance and an increase in the number of interparticle contact points, which enhances powder cohesion and increases incipient shear stress (Hartmann and Palzer, 2011). Incipient shear stresses of T1 and T2 at a given applied normal stress were similar. T0 powders had higher incipient stresses than T1 and T2 powders for all grinding frequencies. This difference is more important for B14 powders for which T1 and T2 powders were significantly less cohesive than T0 powders (Table 3). This indicates that T0 powders had poorer flowability than T1 and T2 powders. The poor flowability of T0 powders may be due to their greater abundance of fiber and/or their greater water content, the latter often-enhancing powder cohesion is made possible because of its plasticizing effect. Moreover, Petit et al. (2017) reported higher interparticle contact points in powders rich in rod-shaped particles, such as fiber particles, thus favoring more particle cohesion. T1 and T2 powders, therefore, have better flow properties than T0 powders, which were cohesive and more sensitive to caking. Pearson correlation (R = -0.60) showed a reduction of cohesion and a better flowability of heterogeneous T1 and T2 powders. The fine particles on the surface of the large particles could limit the interparticle contact and friction and improve the flowability; this is the ball rolling effect (Petit et al., 2017). According to Jenike's classification (Table 3), HS_T0_B10, HS_T1_B10, and HS_T0_B14 were cohesive, whereas all other powders felt into the easy-flowing category.

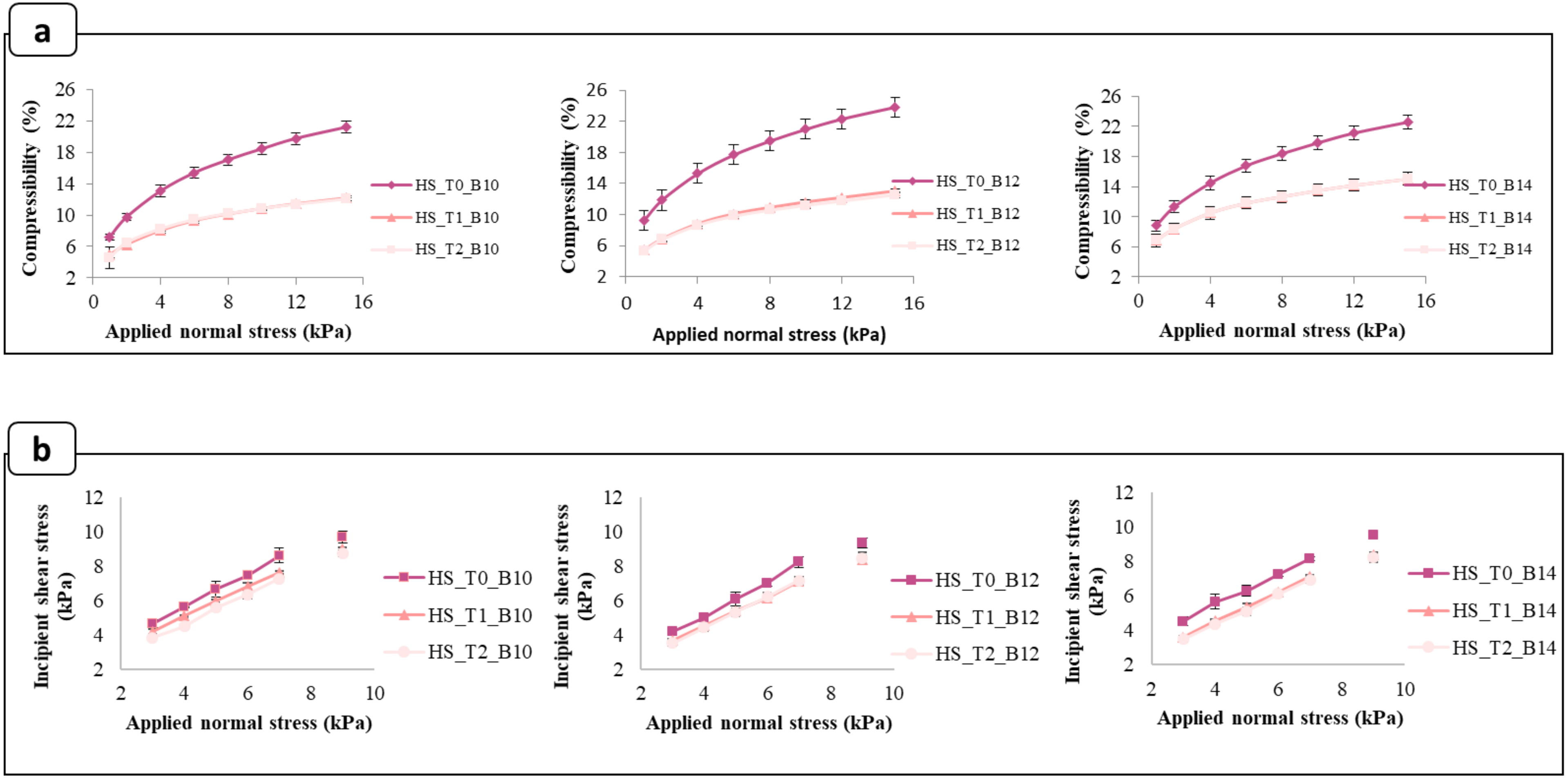


Figure 50: Compressibility (a) and yield locus (b) of *Hibiscus sabdariffa* calyx powders at various applied normal stresses.

4.1.3.2. Impact of processing parameters on reconstituted powders

It can first be observed that an increase in the drying duration enhances the red color intensity of solutions of reconstituted B10 powders (**Figure 51**).

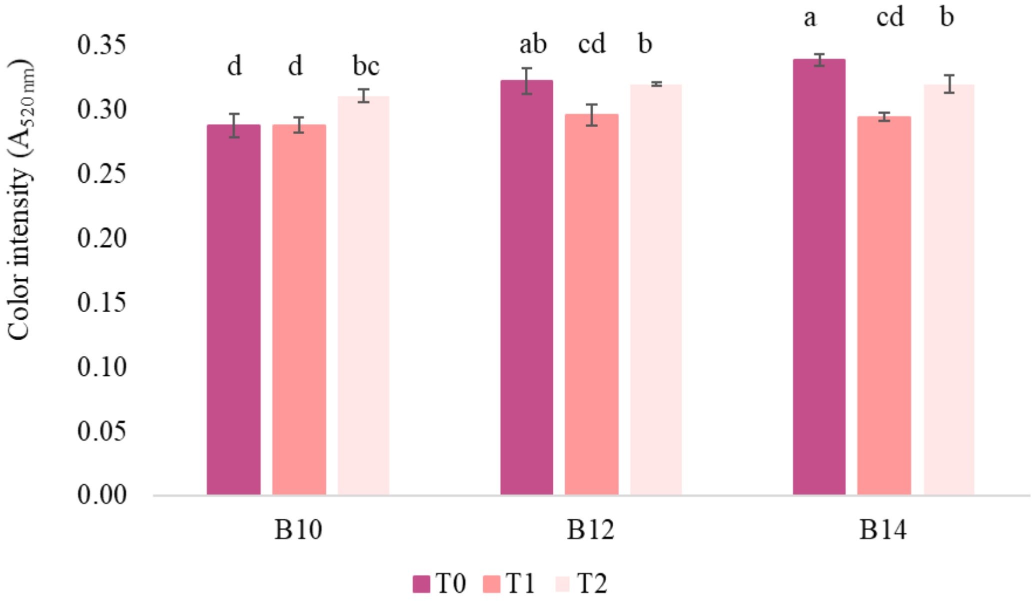


Figure 51: Impact of drying duration and grinding frequency on color intensity (absorbance at 520 nm) of solutions of reconstituted *Hibiscus sabdariffa* powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

For the B10 batch, the color intensity from T2 powder was increased by 8 % compared to T0 powder. This may be explained by the higher acidity of these extracts (**Table 4**), offering better stability of the anthocyanins, therefore, a higher red color intensity (Sinela, 2016) and/or the higher proportion of products of Maillard reaction in powders having undergone a longer drying, as highlighted by chroma values (**Figure 47**). For B12 and B14 batches, the red color intensity of solutions of reconstituted powders increased in the following order: T1 < T2 < T0. The red color intensity was related to the concentration of anthocyanins. Therefore, the anthocyanin concentration of B12 and B14 powders may be decreased during the drying steps. Many studies reported a reduction of *Hibiscus sabdariffa* anthocyanins content during drying or extraction (Chumsri et al., 2008; Daniel et al., 2012; Djaeni et al., 2018; Loyola Arenas et al., 2016; Sinela, 2016). They highlighted a decline of the anthocyanin content with increments of temperature and/or time, thus attracting attention to the thermal sensitivity of anthocyanin molecules during manufacturing.

Table 4: Chemical properties of solutions of reconstituted powders of *Hibiscus sabdariffa* calyxes.

Sample acronym	pH (-)	Brix (°Brix)	Insoluble Material (%) (on wet basis)	Browning index (-)
HS_T0_B10	2.64 ± 0.03 ^{ab}	5.33 ± 0.58 ^{a}	40.25 ± 3.27 ^{ab}	0.45 ± 0.04 ^{a}
HS_T1_B10	2.64 ± 0.01 ^{ab}	5.00 ± 0.00 ^{a}	38.32 ± 0.69 ^{ab}	0.44 ± 0.01 ^{a}
HS_T2_B10	2.59 ± 0.01 ^{b}	5.33 ± 0.58 ^{a}	42.01 ± 1.57 ^{a}	0.44 ± 0.02 ^{a}
HS_T0_B12	2.65 ± 0.01 ^{a}	6.00 ± 0.00 ^{a}	40.27 ± 2.49 ^{ab}	0.47 ± 0.01 ^{a}
HS_T1_B12	2.63 ± 0.00 ^{ab}	5.33 ± 0.58 ^{a}	38.00 ± 0.35 ^{ab}	0.46 ± 0.01 ^{a}
HS_T2_B12	2.61 ± 0.02 ^{ab}	6.00 ± 0.00 ^{a}	38.01 ± 0.96 ^{ab}	0.45 ± 0.01 ^{a}
HS_T0_B14	2.62 ± 0.00 ^{ab}	6.00 ± 0.00 ^{a}	39.55 ± 0.85 ^{ab}	0.44 ± 0.01 ^{a}
HS_T1_B14	2.63 ± 0.01 ^{ab}	6.00 ± 0.00 ^{a}	38.05 ± 0.94 ^{ab}	0.45 ± 0.01 ^{a}
HS_T2_B14	2.62 ± 0.02 ^{ab}	6.00 ± 0.00 ^{a}	36.19 ± 1.03 ^{b}	0.44 ± 0.02 ^{a}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p < 0.05$).

The alteration of anthocyanins should also depend on the characteristics of the matrix in addition to the process parameters. The red color intensity significantly increased with the grinding frequency (Figure 51) for T0 powders only; an increase of 12 % was observed from T0_B10 to T0_B12 powders, and this increase was less significant ($p > 0.05$) for T0_B14 powder, characterized by an increase of only 2 % compared to T0_B12 powder. This trend was confirmed by the Pearson coefficient, indicating a positive moderate influence ($R = 0.51$) of grinding frequency on color intensity. The specific surface area of particles increased during the grinding process (Singh et al., 2018), making powder constituents such as anthocyanins, which are responsible for the red color and more accessible to water, thus improving extraction efficiency. The red color intensity of T1 and T2 powders remained quasi constant when increasing the grinding frequency. It could be due to the drying process, during which the anthocyanin compounds were slightly altered. Moreover, the content of these compounds may have declined over time because of their exposure temperature, oxygen, or light (Deli et al., 2019b; Jackman et al., 1987; Singh et al., 2018; Tham et al., 2018), which leads to the production of browning compounds. The presence of the latter in T1 and T2 powders (Table 4) may be resulting from anthocyanin alteration. As indicated by the Pearson coefficient correlation (R = 0.594), browning compounds were partly responsible for the increase in chroma, therefore for the modification of powder color.

The insoluble material (IM) is presented in Table 4. A non-significant decrease in IM of calyx powders resulted in high drying duration. On the contrary, increasing in grinding frequency from B10 to B14 resulted in a significant reduction of T2 powders IM. Hibiscus calyxes are rich in fiber (Cisse, 2010; Deli et al., 2019b), which makes the grinding difficult (Becker et al., 2016; Zaiter et al., 2016), hence the need for powerful grinding. Important influence of processing parameters on powder IM was obtained due to the particle size reduction favored at higher drying duration and grinding frequency. Brix of reconstituted powders increased significantly only with the grinding frequency from B10 to B14 for T1 powders (Table 4), confirming that higher grinding frequency improves the solubility of reconstituted powder. Powder solubility obtained from finer powders enhanced the availability of components but remained incomplete due to the presence of fiber. This may cause turbidity or precipitation of solutions at rest, which may be an undesirable effect in industry or not be appreciated by consumers.

4.1.4. Additional data

4.1.4.1. Conductivity

The conductivity results presented in Figure 52 allowed to observe the water-soluble molecule extraction. This kinetic is marked by an increasing phase and a stabilization of solution conductivity starting between 50 and 100 s. During the increasing phase, the soluble molecules extraction of T0 powder is progressive, contrary to that of the other powders which is very fast. This progression of the conductivity upon extraction is dependent of the material properties, themselves impacted by the drying time and the grinding frequency. Indeed, longer drying time and higher grinding frequency induce a lower particle size, which is related to a higher specific surface area and thus a higher contact surface between the particles and the solvent. Consequently, the release of soluble molecules is faster and observed at the very beginning of the reconstitution process.

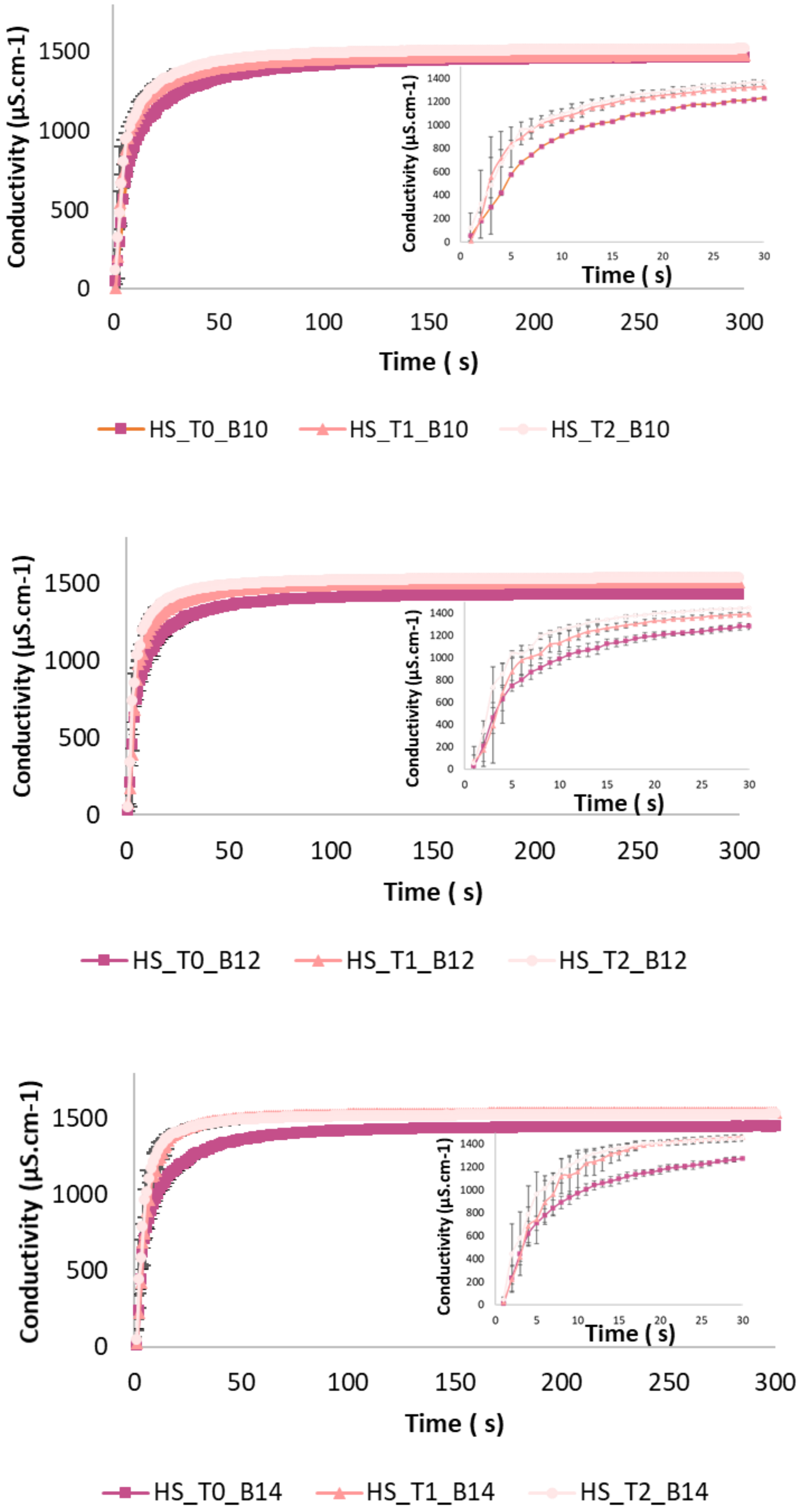


Figure 52: Conductivity kinetics of *Hibiscus sabdariffa* powders in water at 50 °C. Inserts represent the data between 0 and 30 s.

For powders with identical particle sizes, especially the T1 and T2 powders, the conductivity curve tendencies are similar. Most of the powders obtained from the additional drying present the lowest powder reconstitution times (16-20 s) (**Figure 53**). Such powders allow to reduce preparation and industrial production times.

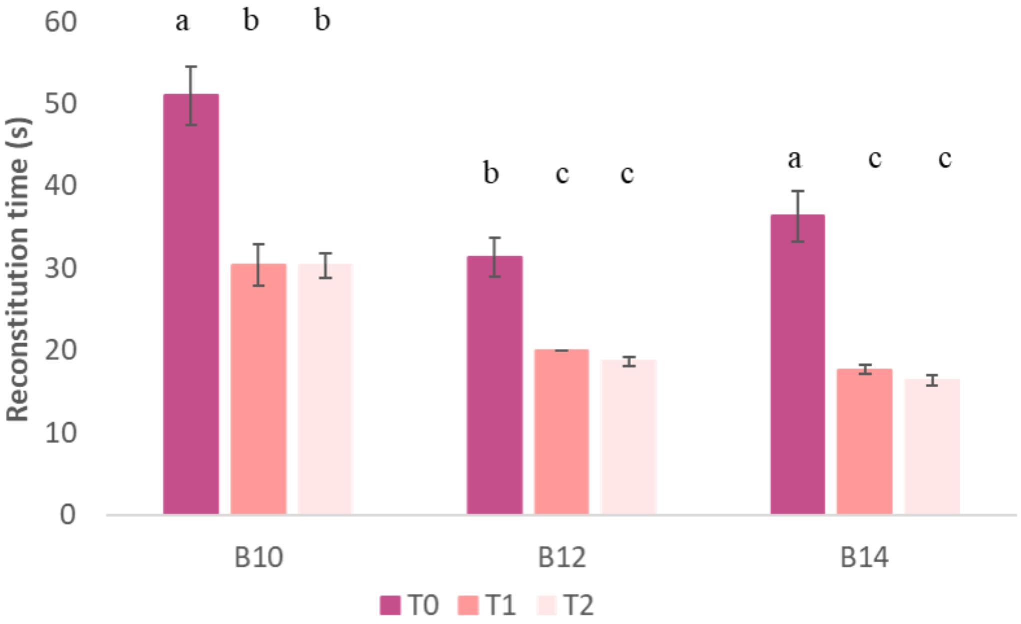


Figure 53: Reconstitution time of *Hibiscus sabdariffa* powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$).

4.1.4.2. Identification of the most influencing properties of hibiscus powders by Principal Component Analysis (PCA)

The F-axes (factors) of the PCA shown in **Figure 54** are linear combinations of variables (physical, chemical and flow properties). The selected F1 and F2 axes represent 78.24 % of the variability in the information, showing the good quality of the PCA representation. The correlation circle (**Figure 54**-a) corresponds to a projection of the variables in a two-dimensional plane consisting in the two factorial axes F1 and F2. F1 and F2 axes in **Figure 54**-a are related to drying time and grinding speed, respectively, in addition to inherent product properties. The F2 axis expresses only physicochemical properties linked to coloration, unlike F1.

According to the F1 axis, the negative correlation between the drying time and the powder physicochemical and functional properties (size, compressibility, cohesion, reconstitution time, insolubility), and the positive correlation between these latter properties, highlights and confirms the following points:

- Increasing the drying time favors the reduction of water content and water activity;
- Lower water content facilitates the grinding of calyxes into fine particles;
- Low water content limits the plasticizing effect of water, which reduces interparticle cohesive forces, compression sensitivity and allows good powder flowability;
- Long drying time causes the increase in chroma values resulting from a browning effect and a modification in powder color;
- Powder reconstitution time is slowed down for powders with high water content. It is likely that the increased water activity changes the particle structure and slows down the water absorption upon reconstitution.

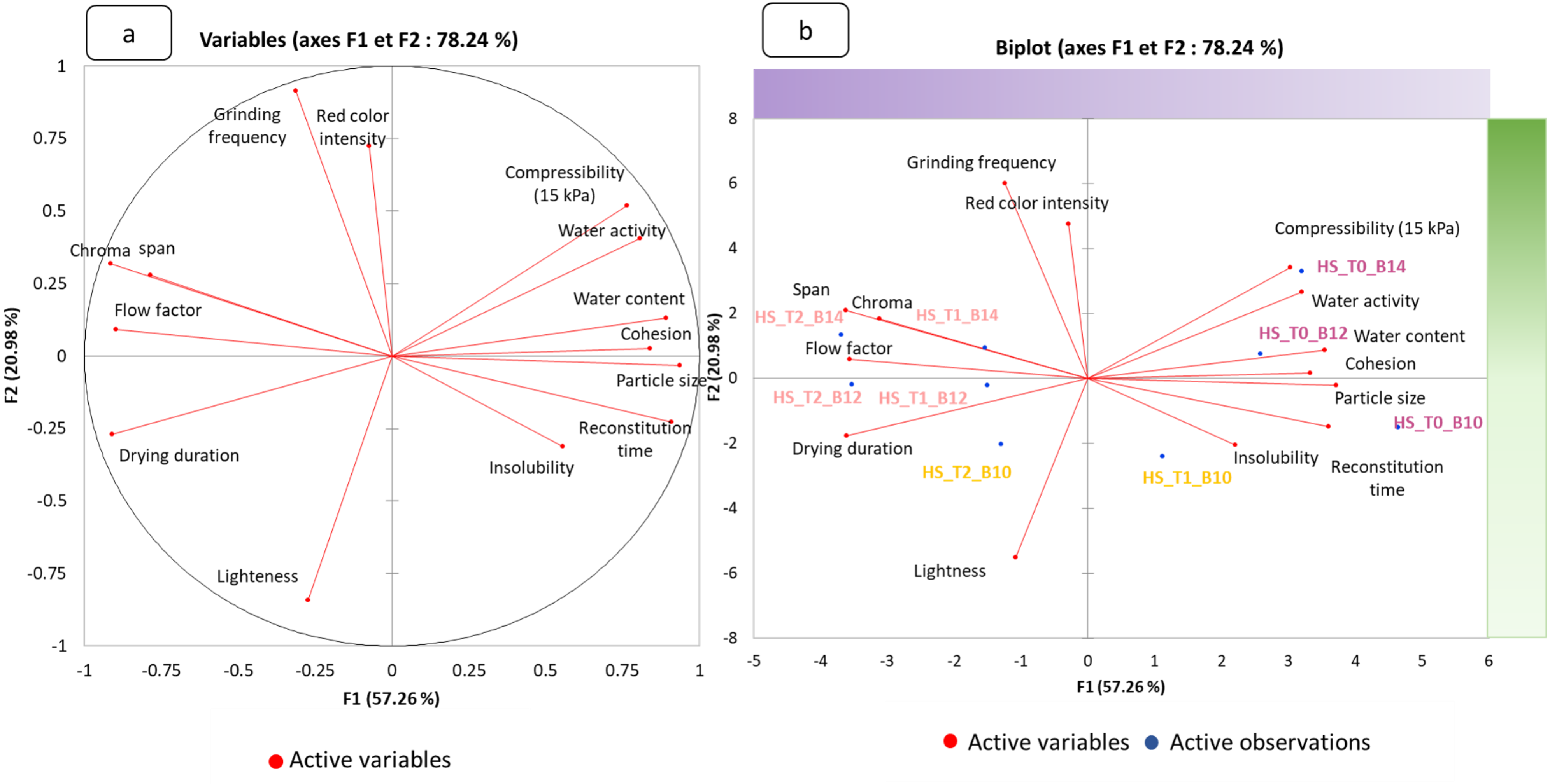


Figure 54: Principal component analysis on *Hibiscus sabdariffa* powders: physicochemical and functional properties; a - correlation circle, b - representation of powder sample distribution.

According to the F2 axis, the positive correlation between grinding frequency and coloration intensity shows the positive effect of powder processing on the accessibility of coloring molecules.

The powders in Figure 54-b are on the one hand, grouped according to the processing parameters: drying time in purple, grinding frequency in green and on the other hand, according to the properties characterizing the powders. The powder distribution on the axes shows the predominant influence of the drying time on the majority of powder properties, while the coloration depends on both the drying and the grinding speed. The powders written in plum color, are composed of large particles, mainly rich in water (short drying time), resulting in a poor powder flowability. However, they produce intense red-color solutions. The yellow group of powders is characterized by low but brilliant red colorations. The salmon pink group consists of powders with low water content (long drying time), therefore improved shelf-life. The powder fine particles promote an easy flowing and a medium intensity red coloring although affected by browning.

This first part demonstrates that the proper adjustment of drying time and grinding is important for the production of *Hibiscus sabdariffa* calyx powders, especially for the preservation of the anthocyanin content and the powder flowability. These features are important end-use functionalities for consumers and decisive in the purchase process of such a product. This statistical analysis allowed the identification of powder categories and their determining properties.

4.1.5. Conclusion

Water content, fiber rate, particle size and shape, heterogeneity, and cohesion are relevant to understand the flowability of hibiscus powders, induced by drying coupled with grinding. Complementary drying times to sun-drying allow the production of almost identical powder with good rheological behavior. Despite this good flowability, it is important to note that the anthocyanins may be altered at high drying duration, particularly in fine powders where specific surface area is more important. Therefore, the combined effect of drying and grinding on powders leads to the right adjustment of drying duration and grinding when powdering *Hibiscus sabdariffa* calyxes because of the preservation of its quality. On this basis, powders T1_B12, T1_B14, T2_B12, and T2_B14 are of particular interest because they combine good solubility, short reconstitution time, easy flowing, have a relatively good red color, and have a low water content to ensure a longer shelf-life. In the following section, in addition to the red color evaluation, the anthocyanin content and the antioxidant activity have to be assessed to ensure the nutritional quality of the powders.

Introduction à la seconde partie des résultats

Outre les propriétés physicochimiques et d'écoulement, un intérêt est particulièrement porté sur l'étude du comportement des poudres pendant leur reconstitution en milieu aqueux. En effet, pour la préparation de boissons, les poudres d'hibiscus sont ajoutées à une quantité d'eau et infusées pour une durée donnée. La durée de cette étape est importante puisqu'il s'agit notamment du passage en solution des biomolécules d'intérêt.

Ainsi, la seconde partie des travaux porte sur l'étude approfondie de la reconstitution des poudres de calices séchés et broyés dans l'optique d'une valorisation de leurs fonctionnalités. Plus précisément, il est question d'identifier les étapes de la reconstitution des poudres d'*Hibiscus sabdariffa* et d'en déterminer l'impact sur la capacité de libération des biomolécules.

La granulométrie des poudres obtenues précédemment étant polydisperse, un tamisage a été effectué et des caractérisations physicochimiques des fractions ont été effectuées ainsi que l'évaluation de leurs propriétés fonctionnelles (écoulement et reconstitution). Sur la base de ces analyses, une meilleure valorisation des fractions de poudres d'hibiscus a pu être proposée notamment par la prise en compte de la teneur en anthocyanes.

En effet, les calices d'*Hibiscus sabdariffa* sont riches en anthocyanes et en polyphénols, ce qui leur confère des propriétés antioxydantes intéressantes. La production de poudre de calices et le fractionnement par tamisage améliorent la capacité d'extraction de ces biomolécules et permettent de produire des poudres présentant des propriétés fonctionnelles ciblées (meilleurs écoulement et capacité à se reconstituer, faible activité de l'eau assurant une longue durée de conservation). Dans le but d'élucider les étapes de la reconstitution des poudres, la combinaison de méthodes telles que la morphogranulométrie, la conductimétrie et la microscopie ont été employées pour caractériser les évolutions de la taille et de la forme des particules au cours de la reconstitution et établir leur lien avec la libération des biomolécules.

Pendant la reconstitution, la diffusion de l'eau dans les particules entraîne une solubilisation rapide des molécules polaires, un gonflement continu et une dissociation des particules, suivie d'une fragmentation. Les cinétiques de ces phénomènes sont étroitement liées aux caractéristiques physiques (taille, forme et structure), aux forces interparticulaires et à la composition chimique des poudres. Les particules de petite taille (11 - 85 µm) et de structure poreuse favorisent la pénétration de l'eau facilitant le gonflement et la dispersion des particules et par conséquent l'extraction des anthocyanes en est améliorée. De plus, ces poudres fines sont enrichies en molécules bioactives, ce qui leur confère un pouvoir antioxydant plus élevé que les poudres grossières qui sont caractérisées par leur richesse en particules fibreuses.

Ce travail (**Figure 55**) a fait l’objet d’une seconde publication dans le journal « Powder technology » :

M'be, C. U., J. Scher, J. Petit, C. Paris, N. G. G. Amani, and J. Burgain. "Effect of powder fractionation on anthocyanin extraction kinetics during powder reconstitution." *Powder Technology* 415 (2023): 118119.

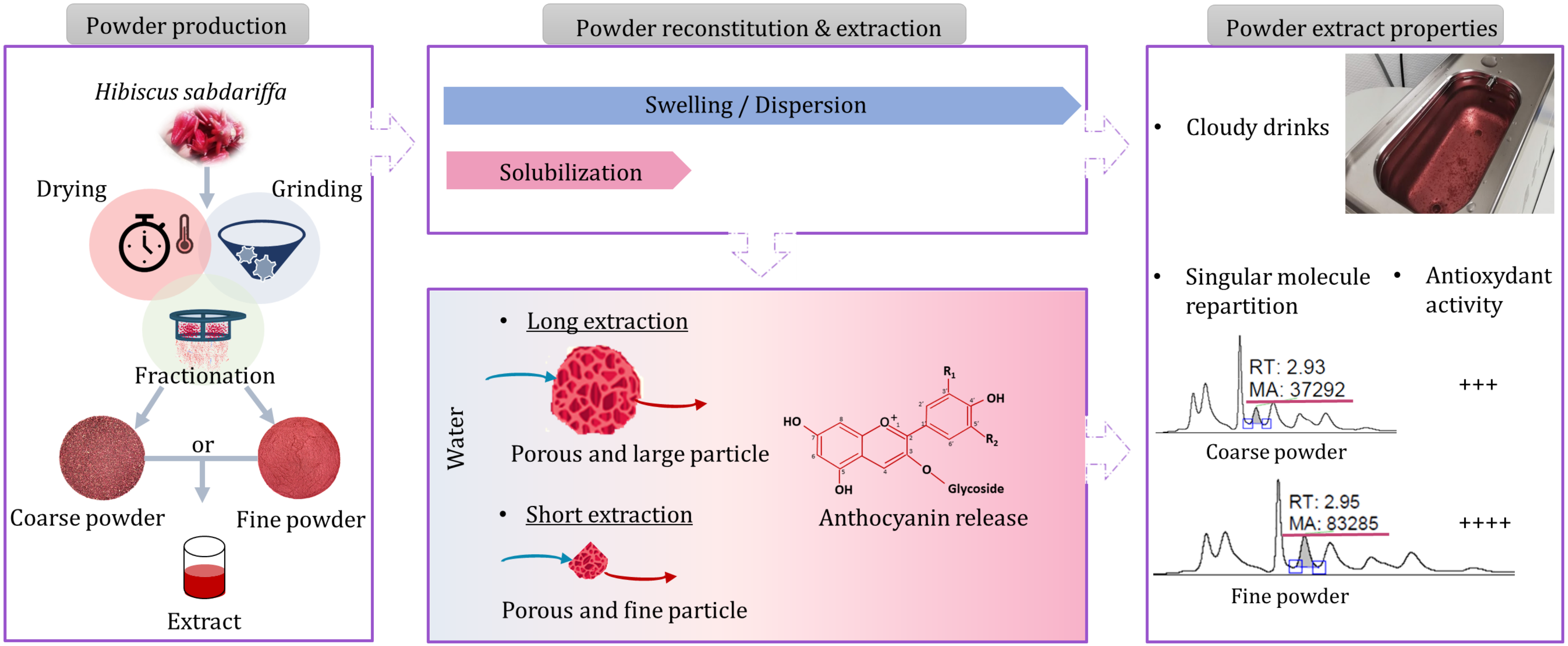


Figure 55: Résumé graphique de la production et de la reconstitution des poudres d'*Hibiscus sabdariffa* obtenues par étuvage et broyage.

4.2. Reconstitution mechanism of *Hibiscus sabdariffa* powders and evaluation of antioxidant activity of resulting aqueous solutions: Article n°2

4.2.1. Introduction

Hibiscus sabdariffa (hibiscus) calyxes are edible plants, which are considered as attractive natural colorants and have a real health potential, hence their use in food, cosmetics, and medical sectors (Cisse, 2010; Hirunpanich et al., 2006; Pérez-Torres et al., 2013). Regarding its manufacturing, the challenges for the food industry are to be able to adequately respond to consumer demands, reduce food waste, provide good preservation abilities to food products, and make them accessible. To this end, several unit operations have been studied and conclusive results were obtained. Post-harvest drying is very useful to overcome the problem of seasonality, it also facilitates transportation and thus allows worldwide access to this tropical plant (Builders et al., 2010; Kumar et al., 2015; Marnoto, 2014; Tham et al., 2018). For various reasons, including the improvement of consumer satisfaction, aqueous extracts of dried calyxes or even fresh calyxes (infusions, fresh drinks) have also been produced. These ready-to-use products are considered as sensitive due to the instability of anthocyanins at high water activity (Sinela, 2016). The anthocyanin alteration results in the decrease in health benefits and discoloration of beverages that reduces their interest and attractivity. The production of instant powders from hibiscus juice may be a way to limit this anthocyanin degradation, since the water activity is lowered. From another point of view, powdering improves functionalities of hibiscus calyxes by increasing the bioavailability of its components, as well as antioxidant activity (Cisse, 2010). The transformation into powder also facilitates handling and storage by markedly reducing the volume. However, the main issue of hibiscus powder production is the thermal sensitivity of anthocyanins, which is strongly related to process conditions (Cheng et al., 2014; Hu et al., 2012; Tham et al., 2018). In practice, optimums are sought to limit the loss of anthocyanins and polyphenols of hibiscus. Hibiscus calyx powders are mainly produced by two methods: spray-drying and grinding. The production of powders by spray-drying requires prior pretreatment involving several unit operations. Harvested calyxes are usually first stabilized by drying. After that, liquid extraction of soluble compounds from dried calyxes is carried out and the obtained extract is concentrated then spray-dried to obtain easy-to-use instant powders. However, the thermal pretreatments and spray-drying alter bioactive molecules of hibiscus and consequently reduce the antioxidant activity (Cassol and Noreña, 2021; Deli et al., 2019b; C. A.

Monteiro et al., 2019). A second powdering method proposes a shorter production line that requires less thermal treatments: the combination of fresh calyx drying with grinding. Drying here plays a double role by reducing water activity: improving microbiological quality therefore the shelf-life and facilitating grinding by providing friable calyxes. Certainly, this treatment results in a powder rich in insoluble material, but presents some advantages. This may be the most suitable method for the preservation of bioactive compounds, provided that the heat treatment induced by the drying step and in a lesser extent by the grinding step is controlled. Such a process is a way to obtain powders considered as minimally processed food according to the NOVA food classification system (C. A. Monteiro et al., 2019), which are increasingly in demand by consumers. The classification system called NOVA divides foods into four groups according to the objectives and the transformation degree of food products: unprocessed or minimally processed foods, processed culinary ingredients, processed, and ultra-processed foods (C. A. Monteiro et al., 2019). Indeed, the minimal processing is known to improve product stabilization upon storage while preserving their nutritional and sensory properties (C. A. Monteiro et al., 2019). One of the advantages of the powder form is the convenience and ease-of-use for consumers (e.g., hot or cold drinks preparation, food or cosmetics coloration, etc.). The uses of hibiscus powders (infusion, juice, jam, wine, beer, etc.) often requires to put them in an aqueous solution (Gaiani et al., 2005; Hogekamp and Schubert, 2003; Kravtchenko et al., 1999; Lamiot et al., 1998; Selomulya and Fang, 2013), hence the interest to study their reconstitution (Fang et al., 2008; Selomulya and Fang, 2013; Wu et al., 2008). The reconstitutability of a powder could be an indicator of production process performance and product quality. Indeed, the properties of a powder-based product, as well as the duration and the cost of its preparation, depend on the ability to reconstitute powdered ingredients (Saggin and Coupland, 2002; Selomulya and Fang, 2013). Powder reconstitution occurs in different steps: wetting, sinking/swelling, dispersion, and solubilization (Fang et al., 2008). Wetting is the phenomenon during which the liquid gradually replaces the gas phase at the particle surface after that the liquid was put into contact with particle surface (Fournaise et al., 2021b, 2020; Selomulya and Fang, 2013). If sinkability has often been confused with wettability (Selomulya and Fang, 2013), some authors have clearly defined sinking as the phenomenon during which water diffuses into the particle, increasing its density, which enables the particle to overcome the surface tension at the particle-liquid interface (Fournaise et al., 2022b, 2021b, 2020; Gaiani, 2006). Simultaneously, the liquid penetrates the particles and results in an increase in particle size; it is the swelling step. Swelling is also marked by a local increase in viscosity of the liquid surrounding the particle which would indicate a softening of particle surfaces. Then, upon dispersion, the mother particles (agglomerates or not) split into several daughter particles (Selomulya and Fang, 2013). Finally, soluble parts of particles dissolve in the solution. These steps are decisive in the overall reconstitution process and evaluation of product functionality (Bhandari Bhesh et al., 2013; Fournaise et al., 2021b; Selomulya and Fang, 2013). The kinetics of these reconstitution steps depend on the intrinsic characteristics of the powder, such as chemical composition (contents in water, carbohydrates, lipids, proteins, fibers, and minerals) and physical properties (e.g., porosity, particle size distribution, shape, and particle structure) (Burgain et al., 2016; Deli et al., 2019b; Fournaise et al., 2020; Selomulya and Fang, 2013), and extrinsic factors, including the nature of solvent, temperature, and stirring conditions (Gaudel et al., 2022; Mitchell et al., 2015; Tan and Sulaiman, 2020). Studies conducted on the reconstitution of hibiscus powders obtained by spray-drying highlighted the influence of drying temperature and carrier agents (e.g., maltodextrin, whey protein isolate, and polydextrose), used to encapsulate the bioactive components, on the reconstitution kinetics (Cassol and Noreña, 2021; Díaz-Bandera et al., 2015; Gonzalez-Palomares et al., 2009; Osman and Endut, 2009b). These studies showed that the release of polyphenols from spray-dried powders in aqueous medium, driven by diffusion, was limited by the presence of carrier agents. Indeed, the latter retain the biomolecules by intermolecular interactions (e.g. hydrogen interaction) and consequently reduce the solution maximal concentration, thus increasing the extraction duration (Díaz-Bandera et al., 2015; Nadal et al., 2016). This kind of interactions is often desired in medical and health food fields to avoid their loss in the gastrointestinal tract and ensure that the desired biomolecule concentration reach the target tissue. Moreover, having assessed the dispersibility and solubility of grinding-induced plant powders, (Deli et al., 2019b) showed a better dispersibility and solubility for fine powders due to their greater specific surface area than that of coarse powders. Particle size distribution of powder is therefore a key parameter to master, not only to improve their reconstitutability but also their flow properties. Indeed, an undesirable change in particle size distribution can occur during storage: upon increase in temperature and/or water content, the particles may become rubbery as a result of glass transition. Consequently, particles are susceptible to adhere to each other and form agglomerates or even cake (leading to the formation of a solid block). This causes poor flowability of powders but also delay their reconstitution time due to interparticle cohesion

(Fournaise et al., 2022b; Gaudel et al., 2022). The interest in evaluating the flow properties lies in the control of handling and storage conditions to maintain a better flowability of powders from processing to consumption. The challenge is to obtain powders that are both easily reconstitutable and have good flowability, considering the possible interaction between these two properties. Thus, the present study aimed at characterizing the physicochemical features of *Hibiscus sabdariffa* calyx powders obtained by successive drying, grinding and fractionation and linking them to their functional properties (flowability, reconstitutability). The study was further focused on the elucidation of the reconstitution mechanism, with the ultimate goal to link reconstitution properties to anthocyanin release and antioxidant activity of the extract.

4.2.2. Materials and methods

Material (see section 3.1.1 – First calyx harvest)

Powder production (see section 3.2.1 and 3.2.3)

On the basis of good solubility, good flowability, richness in colored compounds, and low water content (M'be et al., 2022), the HS_T1_B14 (1 h drying, 14 000 rpm), HS_T2_B12 (2 h drying, 12 000 rpm), and HS_T2_B14 (2 h drying, 14 000 rpm) powders were selected.
These three powders were then fractionated by sieving (212 um mesh) to obtain fine (F) and coarse (C) powders.

Powder physicochemical characterization (see section 3.3)

- Water content and water activity
- Color properties
- Particle size distribution
- Particle morphology
- Scanning electron microscopy
- Morphogranulometry
- Proportion of fibrous particles
- Powder flowability

Powder extracts analysis (see section 3.4)

- Anthocyanin content and antioxidant activity
- Color of extracts
- Anthocyanins content determination
- High performance liquid chromatography coupled to mass spec analysis
- Antioxidant activity (PAOT Liquid Technology)

Powder reconstitution (see section 3.5)

- Reconstitution followed by morphogranulometry and microscopy
- Reconstitution monitored by conductimetry

Statistical analysis (see section3.6)

4.2.3. Results and discussion

4.2.3.1. Particle size distribution and presence of fibrous particles

Granulometric characteristics and proportion of fibrous particles are presented in Table 5. Fine powders presented similar average particle sizes (between 85 and 111 µm) and it was the same for coarse powders (median particle sizes between 436 and 492 µm). Span values were well different for granulometric fractions: fine powders were characterized by wider size distributions with markedly higher spans than coarse powders, indicating the higher heterogeneity of fine fractions. Following a decreasing order of fine powder heterogeneity, i.e. span values, powders were ranked as follows: HS_T2_B12_F > HS_T2_B14_F > HS_T1_B14 _F.

Table 5: Granulometric characteristics, fiber content, and flowing properties of *Hibiscus sabdariffa* powder fractions.

Powder sample	D_{50} (µm)	Span (-)	Proportion of fibrous particle (fiber number / 100 g powder)	Compressibility at 15 kPa (%)	Cohesion (kPa)	Flow factor (-)
HS_T1_B14 _F	111.0 ± 1.00 ^{b}	2.20 ± 0.01 ^{b}	0.10 ± 0.00 ^{d}	18.39 ± 0.97 ^{a}	0.83 ± 0.14 ^{a}	5.18 ± 0.72 ^{a}
HS_T2_B12_F	85.9 ± 5.66 ^{b}	2.94 ± 0.35 ^{a}	0.11 ± 0.00 ^{d}	18.63 ± 0.82 ^{a}	0.69 ± 0.01 ^{a}	6.46 ± 0.07 ^{a}
HS_T2_B14_F	90.6 ± 0.57 ^{b}	2.52 ± 0.02 ^{ab}	0.08 ± 0.00 ^{d}	17.57 ± 0.15 ^{a}	0.70 ± 0.19 ^{a}	6.57 ± 1.38 ^{a}
HS_T1_B14 _C	492.3 ± 34.53 ^{a}	1.66 ± 0.16 ^{c}	0.63 ± 0.02 ^{a}	8.88 ± 0.39 ^{b}	0.84 ± 0.06 ^{a}	5.80 ± 0.20 ^{a}
HS_T2_B12_C	485.3 ± 34.79 ^{a}	1.29 ± 0.09 ^{cd}	0.46 ± 0.08 ^{b}	5.58 ± 0.15 ^{c}	0.73 ± 0.10 ^{a}	6.75 ± 0.95 ^{a}
HS_T2_B14_C	436.7 ± 27.68 ^{a}	1.19 ± 0.11 ^{d}	0.31 ± 0.01 ^{c}	5.31 ± 0.57 ^{c}	0.80 ± 0.14 ^{a}	6.36 ± 0.95 ^{a}

Means ± standard deviations with different letters in the same column significantly differed according to Tukey's HSD test ($n = 3$, $p \leq 0.05$).

Deli et al., (2019) reported higher contents in fibers and carbohydrates in coarse hibiscus calyx powders. This was also evidenced by Waiss et al., (2020) in their work on the fractionation of okra seed powders. The results of chemical analyses of fiber content in these studies match the results obtained by morphogranulometry in the current study: the proportion of fibrous particles in fine powders were lower than those in coarse powders (Table 5). The majority of fibers were retained by the sieve during powder fractionation on the basis of particle size. However, the fine powders also contained fibers. Indeed, during the fractionation, the vertical vibrations caused changes of particle orientation and facilitate the passage of the fines as well as the fibers through the sieve of 212 µm mesh size. This presence of fibers in the fine powders would contribute to their heterogeneity, in addition to the particle diversity induced by the grinding process (M'be et al., 2022).

4.2.3.2. Powder flowability

Powder flow properties of hibiscus powder fractions are also displayed in Table 5. Fine powders had identical compressibility values, ranging from 17 % to 19 %, which is consistent with their similar granulometric characteristics and proportion of fibrous particles. Compressibility of coarse powders was lower than for fine powders, which may indicate their better flowability. Indeed, fine powders were composed of more small particles, which have a greater contact area, leading to an increase in interparticle forces impairing powder flow (Petit et al., 2017). Similar observations were done by M'be et al. (2022), Petit et al. (2017), Irie et al. (2021), and Gnagne et al. (2017) showing the highest compressibility of fine powders by comparing for example powdered and granulated sugars, or foutou and foufou powders. In addition, the heterogeneity of fine powder fractions favors a greater reduction in interparticle porosity upon application of 15 kPa normal stress. This led to a slightly lower bulk density of fine powders (0.50 ± 0.02 g.mL^{-1}) than that of coarse powders (0.58 ± 0.02 g.mL^{-1}).

Coarse powders were characterized by low compressibilities (< 10 %). The HS_T1_B14 powder was the most compressible among coarse powders, which may be related to its higher water content (Table 6), shape, and heterogeneity. Indeed, water plays a plasticizing effect, favoring a greater sensitivity to deformation of particles (Fitzpatrick et al., 2007; Zafar et al., 2017). This sensitivity of HS_T1_B14 _C to compression was also likely accentuated by its heterogeneity and higher proportion of fibrous particles that are responsible for more interparticle contact points. The sensitivity of fine powders to compression upon application of normal stresses allows recommendations to be made on the handling and storage conditions to be applied to avoid the agglomeration and caking of powders, an undesirable effect for industrials and consumers. Flow factors values range from 5 to 7 sorted powders as easy-flowing according to Jenike's classification (Jenike, 1964), showing their good aptitude to flow.

Table 6: Physicochemical properties of *Hibiscus sabdariffa* powder fractions.

Powder sample	Water content (g / 100 g on wet basis)	Water activity (-)	L* (-)	a* (-)	b* (-)	Chroma (-)	Hue angle (°)
HS_T1_B14 _F	13.29 ± 0.06 ^{b}	0.52 ± 0.00 ^{a}	44.14 ± 0.29 ^{b}	16.58 ± 0.35 ^{b}	2.86 ± 0.07 ^{b}	16.83 ± 0.36 ^{b}	9.78 ± 0.04 ^{a}
HS_T2_B12_F	11.91 ± 0.06 ^{d}	0.47 ± 0.01 ^{c}	45.42 ± 0.14 ^{a}	17.07 ± 0.29 ^{b}	3.01 ± 0.07 ^{ab}	17.33 ± 0.30 ^{c}	10.01 ± 0.10 ^{d}
HS_T2_B14_F	11.58 ± 0.08 ^{e}	0.43 ± 0.00 ^{d}	45.68 ± 0.25 ^{a}	18.36 ± 0.26 ^{a}	3.10 ± 0.04 ^{a}	18.62 ± 0.26 ^{a}	9.58 ± 0.06 ^{bc}
HS_T1_B14 _C	13.88 ± 0.04 ^{a}	0.51 ± 0.01 ^{ab}	41.37 ± 0.01 ^{d}	9.23 ± 0.02 ^{c}	1.29 ± 0.03 ^{d}	9.32 ± 0.02 ^{c}	7.98 ± 0.19 ^{d}
HS_T2_B12_C	12.29 ± 0.04 ^{c}	0.50 ± 0.00 ^{b}	42.26 ± 0.33 ^{c}	9.72 ± 0.59 ^{c}	1.59 ± 0.12 ^{c}	9.85 ± 0.60 ^{b}	9.29 ± 0.25 ^{ab}
HS_T2_B14_C	11.99 ± 0.03 ^{d}	0.43 ± 0.00 ^{d}	42.02 ± 0.08 ^{c}	9.63 ± 0.16 ^{c}	1.30± 0.03 ^{d}	9.71 ± 0.16 ^{c}	7.67 ± 0.08 ^{c}

Means ± standard deviations with different letters in the same column significantly differed according to Tukey's HSD test ($n = 3$, $p \leq 0.05$).

4.2.3.3. Powder color

A link between powder color and particle size was evidenced by colorimetric analysis. Indeed, higher L*, a*, and b* values were obtained for fine powders (Table 6). The greater differences between fine and coarse powders corresponded to red coloration (a*) and chroma, the latter indicating that color saturation was more pronounced for fine particles. Hue angles were close, showing that the main color hue of fine and coarse powders was similar, i.e. red-violet. The high color intensity of fine powders was probably related to a greater presence of colored molecules of hibiscus, especially anthocyanins, and brown molecules resulting from non-enzymatic reactions. The color differentiation is therefore the result of fractionation and seems to indicate a selective distribution of chemical compounds according to particle size.

4.2.3.4. Powder reconstitution and extraction

4.2.3.4.1 Evolution of *Hibiscus sabdariffa* particles upon reconstitution

As powders must wet and sink before being observed with the morphogranulometer, these reconstitution steps could then not be monitored with this technique.

Average particle sizes of fine and coarse hibiscus powders monotonically increased during the whole reconstitution assays (Figure 56).

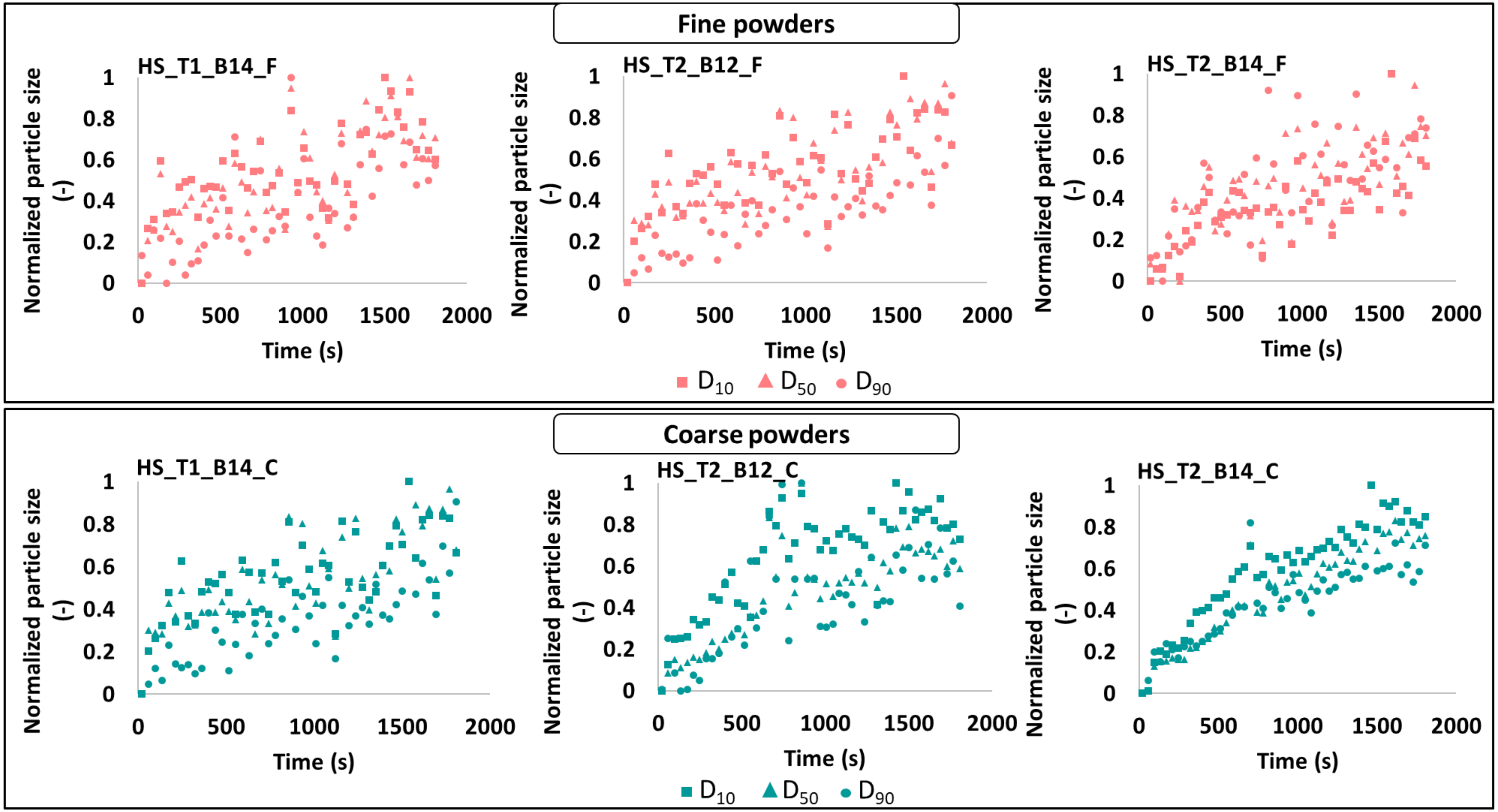


Figure 56: Normalized particle size evolution of *Hibiscus sabdariffa* powders upon reconstitution.

This behavior is typical of powder swelling upon reconstitution and may be related to particle structure, size, and composition. The microscopy images show delimited, empty, and hollow areas on dried coarse powder particles (Figure 57) that were already observed by other authors on ground coffee and were defined as excavated cells delimited by the plant wall cell (Wang and Lim, 2015). This great presence of excavated cells indicates a significant surface porosity of particles (Wang and Lim, 2015).

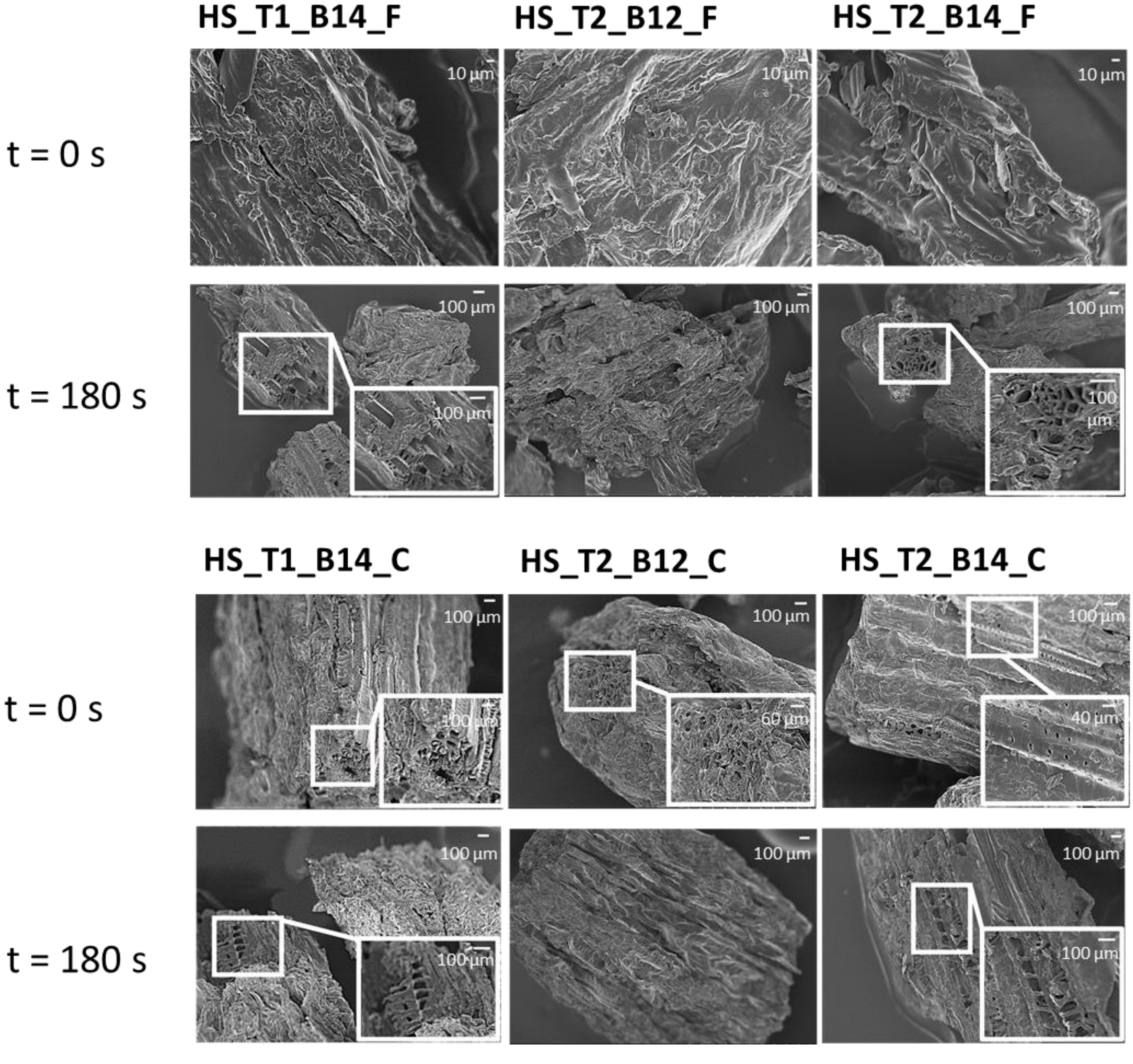


Figure 57: Scanning electronic microscopy images showing the structure of *Hibiscus sabdariffa* particles at dry state (t = 0 s) and after 180 s reconstitution at 500 x, 1 500 x, and 3 000 x magnifications.

The penetration of water in particles occurs at particle surface. Water absorption may be facilitated for smaller particles (fine powders) due to their higher specific surface area. The presence of pores allows water to penetrate the particles likely by capillarity, which facilitates the transfer of water from the surface to the interior of the particle. In fine powders, smaller particles were observed at the surface of large ones preventing the observation of possible open pores in dry state. Another reason may be the destruction of the majority of particle pores during the grinding process.

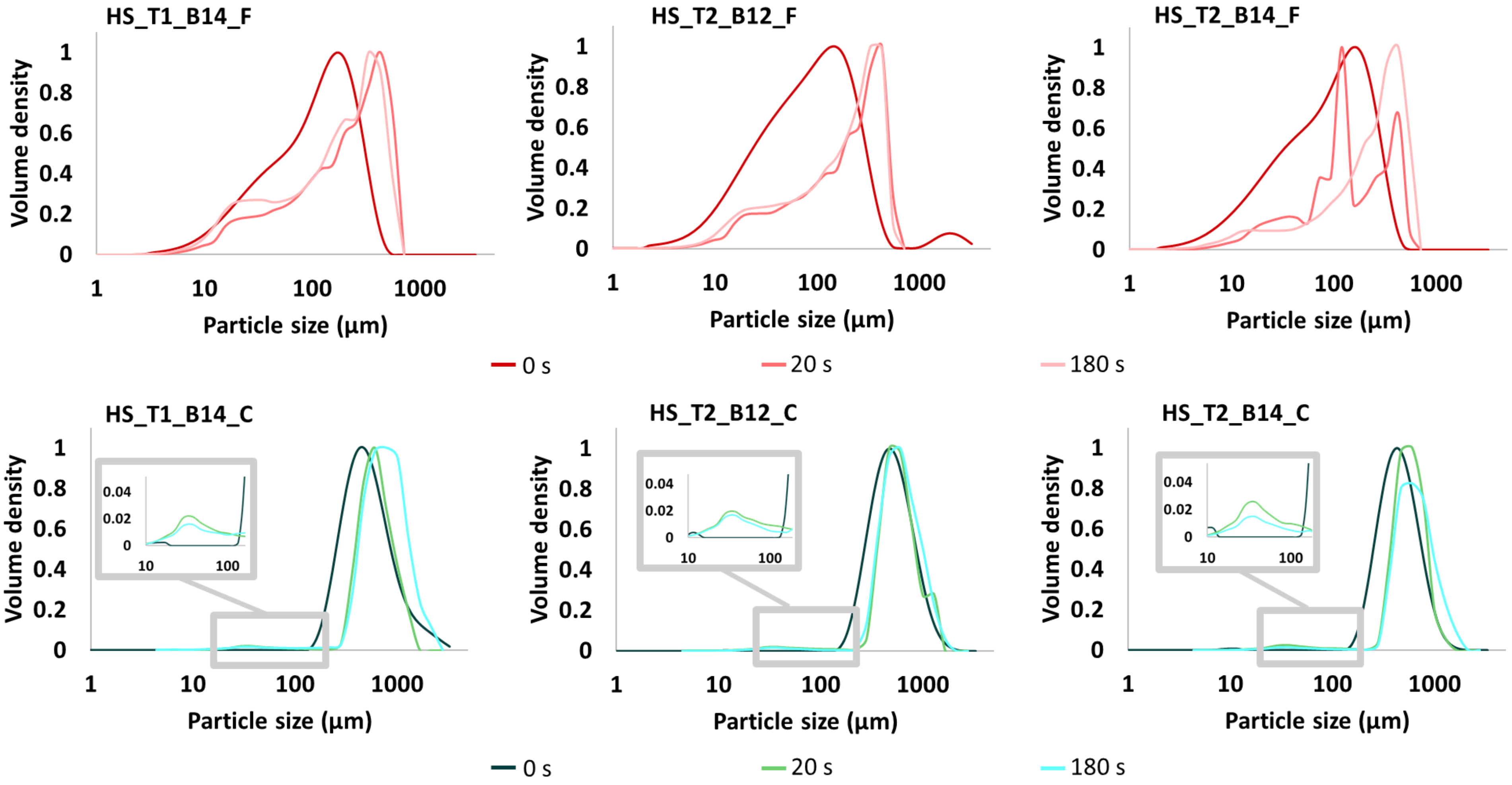


Figure 58: Particle size distribution of fine and coarse *Hibiscus sabdariffa* powders, initially (0 s) and after 20 and 180 s reconstitution time.

Moreover, the high percentage of fibrous particles of cellulosic origin in hibiscus powders could contribute to the swelling behavior evidenced during reconstitution. Indeed, some fibers, for example those of plants, immediately swell upon contact with water which penetrates through their primary walls (Cuissinat and Navard, 2008). At the beginning of reconstitution, the swelling step is also evidenced by a shift of the size distribution curves towards higher particle sizes (Figure 58), and marked by the same order of magnitude of swelling rate for fine and coarse powders (Table 7).

In brief, the swelling of coarse powders is mainly explained by their particle fiber richness and the presence of large pores. As for fine powders, the swelling could be explained by the effect of the particle smallness and fibrous particle content.

Table 7: Reconstitution characteristics of *Hibiscus sabdariffa* calyx fine and coarse powders.

Powder sample	Swelling rate × 10^{-4} (s^{-1})	Dispersion rate × 10^{-4} (s^{-1})		Reconstitution time (s)	Anthocyanin release time (s)
	-	Phase 1	Phase 2	-	-
HS_T1_B14 _F	5.00 ± 1.00 ^{a}	29.70 ± 8.30 ^{a}	2.33 ± 0.58 ^{b}	22.67 ± 0.58 ^{b}	34.13 ± 18.95 ^{c}
HS_T2_B12_F	5.67 ± 2.08 ^{a}	28.67 ± 3.21 ^{a}	3.00 ± 0.00 ^{ab}	20.67 ± 0.58 ^{c}	30.67 ± 10.07 ^{c}
HS_T2_B14_F	3.00 ± 1.00 ^{a}	28.33 ± 4.73 ^{a}	3.33 ± 0.58 ^{ab}	21.00 ± 1.00 ^{bc}	35.00 ± 9.54 ^{c}
HS_T1_B14 _C	6.67 ± 1.15 ^{a}	18.00 ± 2.00 ^{ab}	4.67 ± 0.58 ^{a}	89.46 ± 0.20 ^{a}	416.00 ± 21.17 ^{a}
HS_T2_B12_C	4.33 ± 1.15 ^{a}	12.67 ± 2.08 ^{b}	3.33 ± 0.58 ^{ab}	89.77 ± 0.68 ^{a}	274.67 ± 41.05 ^{b}
HS_T2_B14_C	5.33 ± 1.15 ^{a}	20.00 ± 1.73 ^{ab}	4.00 ± 0.00 ^{ab}	90.13 ± 0.62 ^{a}	277.33 ± 40.27 ^{b}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p \leq 0.05$).

During powder reconstitution, the average particle size and the number of particles (Figure 59) kept increasing: the particles increased in size and some of them dissociated into several particles. The constant increase in the particle number in solution was observed during the whole reconstitution time, evidencing the phenomenon of dispersion. This step took place from the beginning of the reconstitution assay, concurrently with particle swelling. The particle number increase was first rapid from 20 to 150 – 200 s (this first dispersion phase was noted ϕ_1) and then slower (dispersion phase ϕ_2), cf. and Table 7 Figure 59.

Phase 1. This phase is characterized by the fast dispersion of particles (Table 7 and Figure 58). It is the result of dissociation of particles upon contact with water. Indeed, dried particles are often stuck together owing to weak bonds resulting from van der Waals forces and/or solid bridges due to powder chemical composition (sugars in hibiscus for example) (Bhandari Bhesh et al., 2013). The dissociation of stuck particles was evidenced by the results of scanning electron microscopy (Figure 57), the particle size distributions curves of hibiscus powders in dry and wet states (Figure 58), and the particle number increase. In the course of phase 1 (after 20 to 180 s reconstitution), two distinct peaks appear on particle size distributions curves: this shows the presence of two powder populations. Moreover, microscopic observations revealed that fine powders, which had almost no visible pores at their surface in dry state, exhibited some open pores after 180 s reconstitution (Figure 57). These results indicate that in dry state, some small particles adhered to larger ones and obstructed their open pores, leading to agglomerates. The latter disintegrated in water in the dissociation phase, revealing some open pores of large particles and resulting in greater heterogeneity of the particles. In concordance with this study, other authors who studied fibrous particles explained that they dissociate in contact with solvent following the penetration of the liquid in the amorphous regions or porous zones (Cuissinat and Navard, 2008).

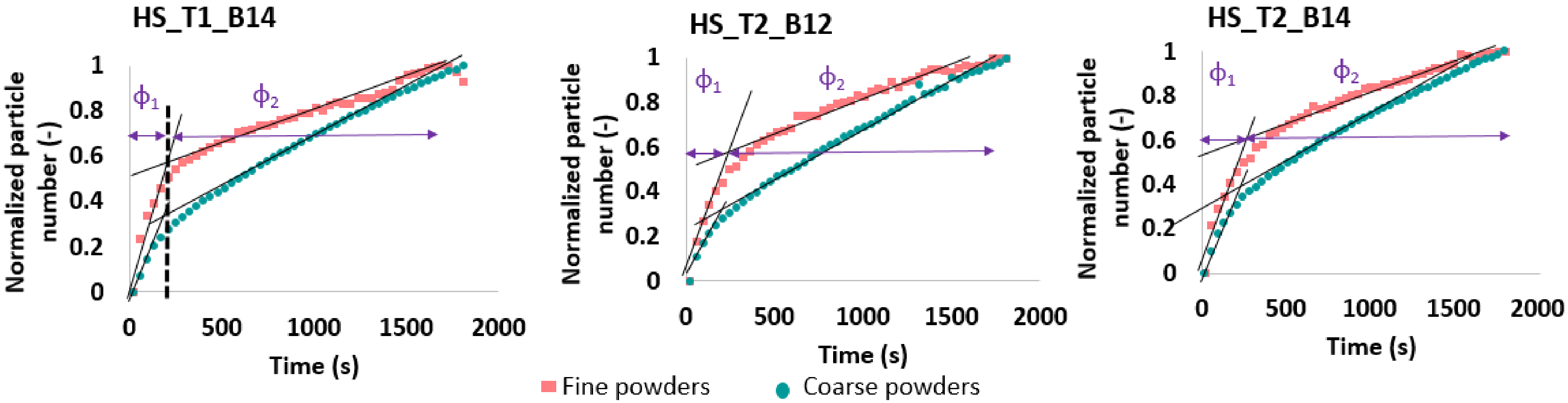


Figure 59: Normalized particle number evolution of *Hibiscus sabdariffa* powders upon reconstitution. φ1 – phase1, φ2 – phase 2.

Phase 2. A slowing down of the dispersion rate of powders characterizes the second reconstitution phase (Table 7). This behavior could correspond to the fragmentation of daughter particles previously released by aggregates. The fragmentation mechanism has been described by Evans and Wilshaw in 1977 (Evans and Wilshaw, 1977) as a process where a particle splits into several smaller parts by the propagation of radial or median cracks (Evans and Wilshaw, 1977; Ghadiri and Zhang, 2002; Han et al., 2021). The high fragmentation rate of the coarser particles can be explained by the penetration of water through the pores, which is probably more important than diffusion through the solid matter.

From these particle number and average particle size increases, it can reasonably be hypothesized that solubilization was hardly observable with the morphogranulometer for this type of powder. Solution conductivity was measured concomitantly with the particle size and shape parameters in reconstitution assays and it is directly deriving from ion release upon powder solubilization. The conductivity of coarse powders increases from 20 to 170 s and then reaches a plateau at 50 $\mu S.cm^{-1}$ (Figure 60). The fine powders, thanks to their high specific surface area, induce a very quick release of ions as they reach the maximum value of 50 $\mu S.cm^{-1}$ from 20 – 25 s (the morphogranulometer and conductivity measurements being carried out from 20 s). The increase in conductivity and apparent red color of the solution proved that the solubilization step quickly took place. The evaluation of reconstitution times (Table 7) from conductivity data shows that solubilization of conductive molecules is faster (by a factor of 4) for fine powders than for coarse powders, which can be explained once again by the higher specific surface area of fine powders. The longer solubilization duration of coarse powders may also be explained by the fact that daughter particles resulting from fragmentation in the course of reconstitution would still contain accessible soluble molecules. In complementary, the difference of solubilization duration between fine and coarse powders could be due to the heterogeneous distribution of soluble molecule in the plant matrix, which in turn induces an unequal molecular distribution within particles.

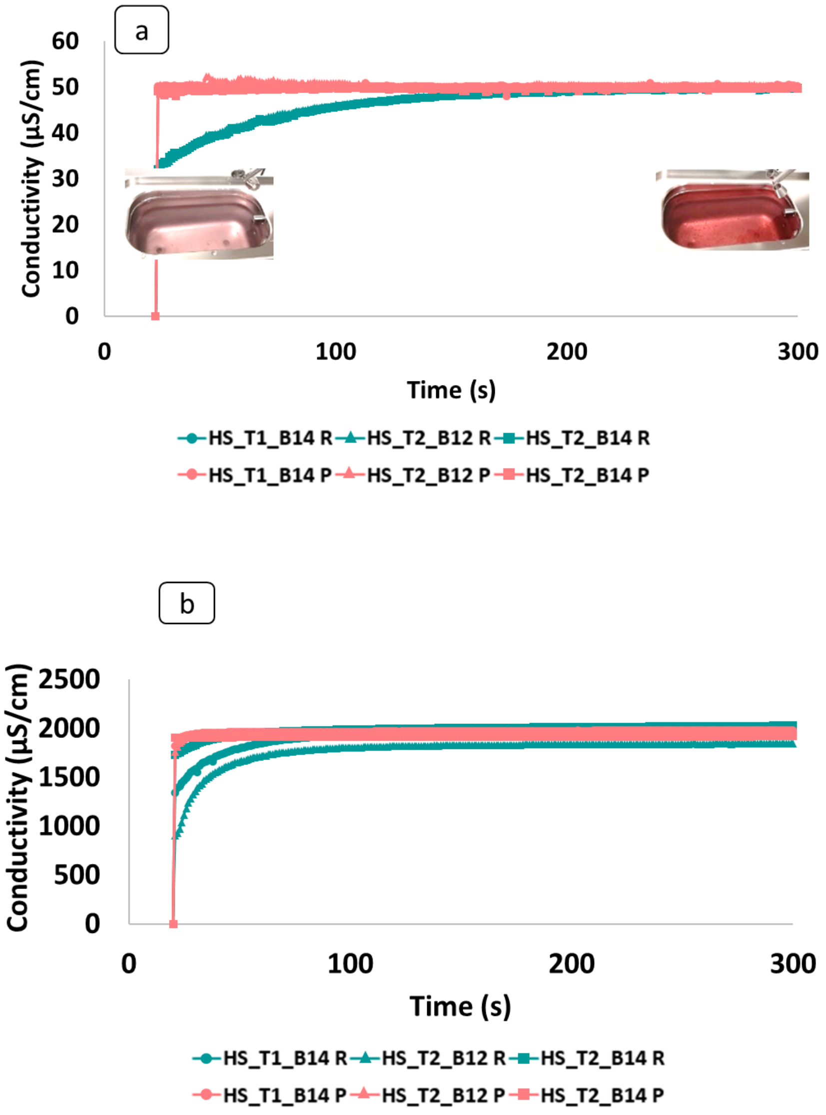


Figure 60: Conductivity evolution of *Hibiscus sabdariffa* extracts at 20 °C (a) and 50 °C (b).

After 30 min reconstitution, the morphogranulometer camera still captured particles in the reconstitution medium. The presence of particles at the end of reconstitution shows that the matrix remained insoluble due to the fiber content and highlights a partial solubilization of all powder fractions.

The proportion of insoluble material was evaluated, showing differences according to powder fraction and extraction temperature. The fine powders were not totally soluble, but their IM at 50 °C (from 38 to 40 %) were lower than those of coarse powders (from 43 to 45 %).

The same trends were obtained at 20 °C, where the insoluble material of fine powders (from 41 to 42 % IM) were also lower than those of coarse powders (from 43 to 47 % IM). The small particle sizes and probably the lower fiber content of fine powders may explain the improvement of their solubility. A too high proportion of insoluble material could make the solutions of reconstituted hibiscus powders cloudy, which can be a deterrent to the consumption of these drinks.

Overall, the constant increase in the particle number and size in solution, the solution conductivity, and the red coloration, evidenced the succession of swelling, dispersion, and solubilization steps of the particles during reconstitution.

4.2.3.4.2 Anthocyanin release kinetics and antioxidant activity

Monitoring the absorbance at 520 nm of aqueous extracts obtained from hibiscus powders in the optimal anthocyanin extraction conditions (50 °C, 30 min) allowed to evaluate the kinetics of their red color intensity (Figure 61). The red color of aqueous extracts of coarse powders gradually intensified up to 150 s and then stabilized. For fine powders, the same trend was observed, but the plateau of absorbance was reached sooner: as early as 20 s. These color stabilization times meet the order of magnitude of reconstitution times deduced from conductimetry, showing that the solubilization step is mainly related to molecules responsible for coloration. This confirmed that fine powders were able to solubilize about 5 to 10 times faster than coarse powders.

Coarse powders required more time for colored compounds extraction, due to their lower specific surface area and their probable different particle structure. Indeed, molecules need to pass through cell walls to be extracted in water and the structure of cell walls begins to be weakened concomitantly with particle swelling. This long extraction time observed for coarse powders may also be due to their composition: i) the components of coarse powders such as fibers would retain more anthocyanins; ii) fine powders are more soluble and therefore the release of colored compounds is easier than for coarse powders where they must escape from a more insoluble matrix.

After 5 min extraction, the absorbances of all fine and coarse powder extracts were identical, underlining that the fractions reached similar red coloration intensities. The same applies to all powder fractions after 30 min extraction. This suggests comparable anthocyanin contents in fine and coarse powder fractions.

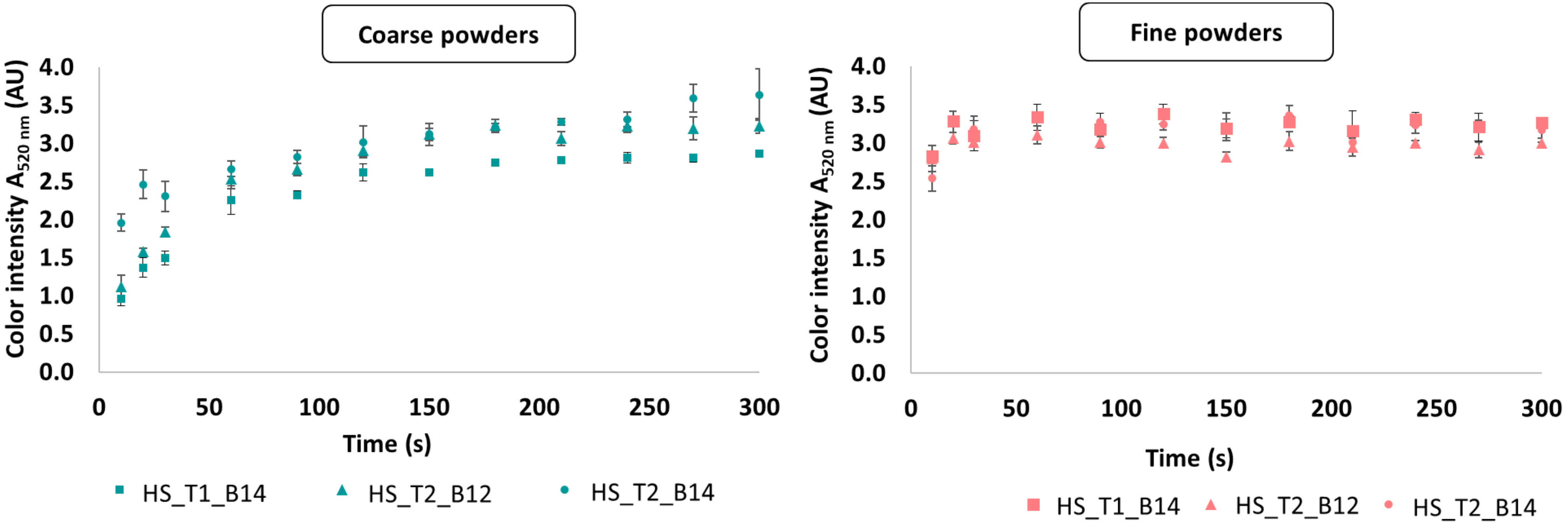


Figure 61: Color intensity evolution of *Hibiscus sabdariffa* extracts at 50 °C.

The release kinetics of anthocyanins in water at 50 °C (Figure 62) followed the same trends as the evolution of the red coloration. For fine powders, the maximal anthocyanin concentration (from 130 to 140 mg.L^{-1} delphinidin 3-xylosylglucoside equivalents) was reached as early as 60 s extraction and then stabilized, which confirms the quick solubilization of anthocyanins. This can be explained by their hydrophilic nature conferring them a very good aptitude for solubilization in polar media such as water and alcohol (Wang et al., 2016). Anthocyanins required more time to be released from coarse powders, about 120 s for HS_T2_B14 _C and 300 s for HS_T1_B14 _C and HS_T2_B12_C, in accordance with what was observed for color intensification kinetics. At this stage, morphogranulometric monitoring evidenced that fine powders were not fully reconstituted, as much insoluble material remained.

The high correlation degree between the anthocyanin concentration of extracts after 30 min at 20 °C and the chroma value of powders (R = 0.85) shows the dominant contribution of anthocyanins on color of extracts. For extraction assays at 50 °C, anthocyanin content and chroma values were less correlated but the Pearson correlation coefficient R of 0.70 still indicated a moderate correlation. This may be explained by the fact that other molecules than anthocyanins may significantly contribute to the powder extract color when increasing temperature, such as biomolecules or/and non-enzymatic browning products that may have been formed during the drying and grinding processes. In addition, molecules resulting from non-enzymatic browning are expected to be preferentially found at the surface of hibiscus particles due to their formation mechanism requiring high temperature and intermediate water content, whereas hibiscus anthocyanins are known to be stored in cell vacuoles (Juhari et al., 2021). Thus, the solubilization of anthocyanins could require more time than molecules resulting from non-enzymatic browning, leading to different kinetics for color intensity and anthocyanin release.

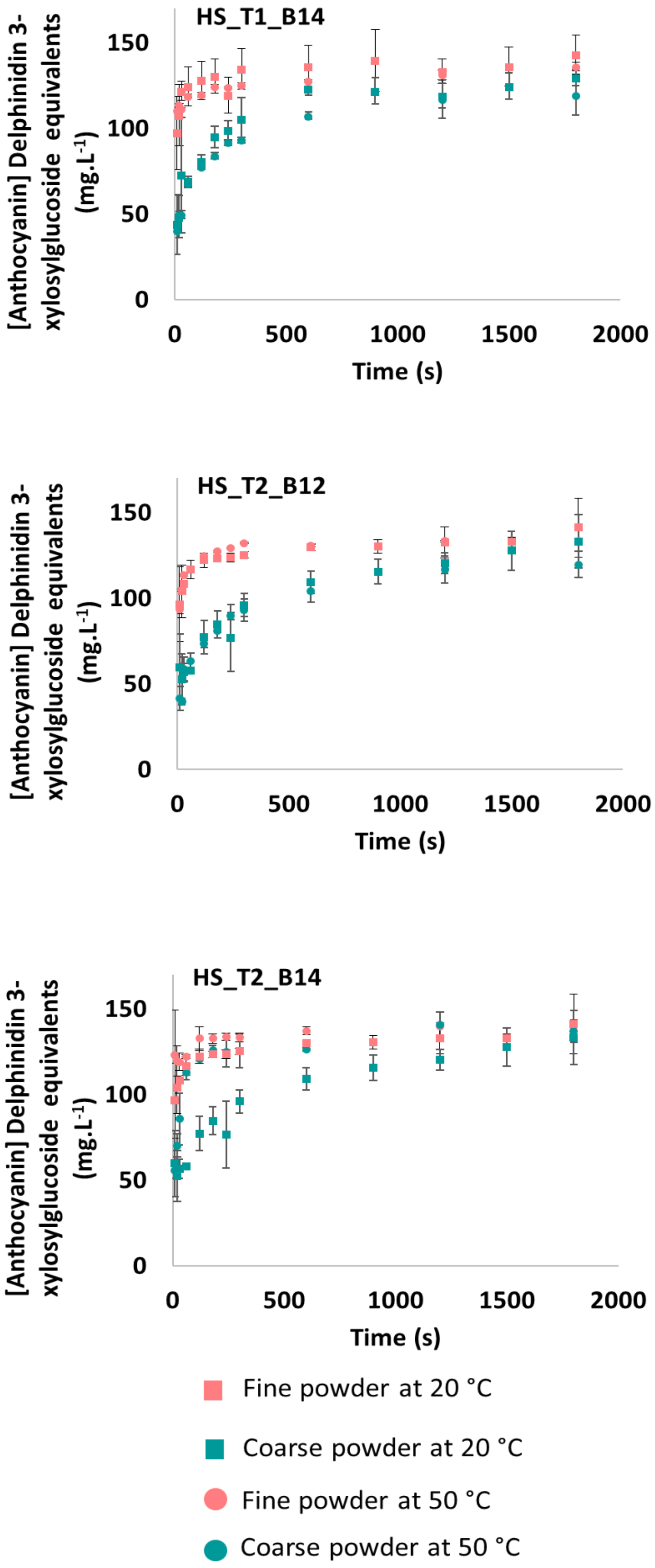


Figure 62: Kinetics of *Hibiscus sabdariffa* anthocyanin release in water.

Previous results have shown that anthocyanin release depends on particle size; it is now interesting to focus on the impact of the reconstitution environment such as water temperature.

The release kinetics of anthocyanins in water at 20 and 50 °C follow the same trends (Figure 62). For fine powders, no significant difference could be attributed to the extraction temperature, extraction curves were almost superimposed. However, the anthocyanin concentrations at 20 °C of the coarse powders stabilized only after 600 s. The difference between extraction kinetics at 20 and 50 °C was more marked for the HS_T2_B14 _C powder: anthocyanin release was faster at 50 °C than at 20 °C. Moreover, the maximum anthocyanin concentration (roughly 130 mg.L^{-1} delphinidin 3-xylosylglucoside equivalents) was identical for all powder fractions and extraction temperatures. This shows that the preparation of drinks from hibiscus powders could be realized either from fine or coarse powders at 20 °C to reach the same anthocyanin concentration than at 50 °C without much increase in extraction time, while limiting the possibility of heat-induced degradation of these antioxidants. Fine powders present the advantages to provide faster extraction of soluble material, which can be interesting for consumers and industrials. Whatever the powder fractions, the anthocyanin release times were greater than reconstitution times because the same stirring speed (650 rpm) but different powder-to-water ratios were used for reconstitution and anthocyanin extraction assays.

Extraction results evidenced similarity of anthocyanin contents of fine and coarse fractions of all types of powders (HS_T1_B14, HS_T2_B12, and HS_T2_B14). However, the antioxidant activity of HS_T2_B14 powders (used as models because they contain the lowest water activity, thus this let expect a longer shelf-life), was significantly higher for the fine powder (289.40 ± 1.93 PAOT score / g product) than for the coarse powder (207.59 ± 4.96 PAOT score / g product). Powder fractionation by sieving led to a concentration of antioxidant molecules (polyphenols, anthocyanins) in the fine powders. The chromatograms of powder extracts (Figure 63) showed a very close composition of extracts obtained from fine and coarse powder fractions, except for one compound eluted at about 2.95 min retention time, which appeared much more concentrated in fine powder extracts. This unknown molecule of molar mass of circa 358 g.mol^{-1} (m/z = 357 in ESI^{-}), is close to the 5-caffeoylquinic acid (m/z = 353 in ESI^{-}), an antioxidant (Fadhil et al., 2021; Piovesana et al., 2019), and could be the reason for the higher antioxidant activity of fine powders. This last result highlights the interest of applying fractionation by sieving, which would allow the valorization of the functionalities of powder fractions and optimize their end-uses. These fractions could be used for different purposes (infusion, quasi-instant drink, coloring agent), while benefiting from the same coloring intensities or similar anthocyanin contents.

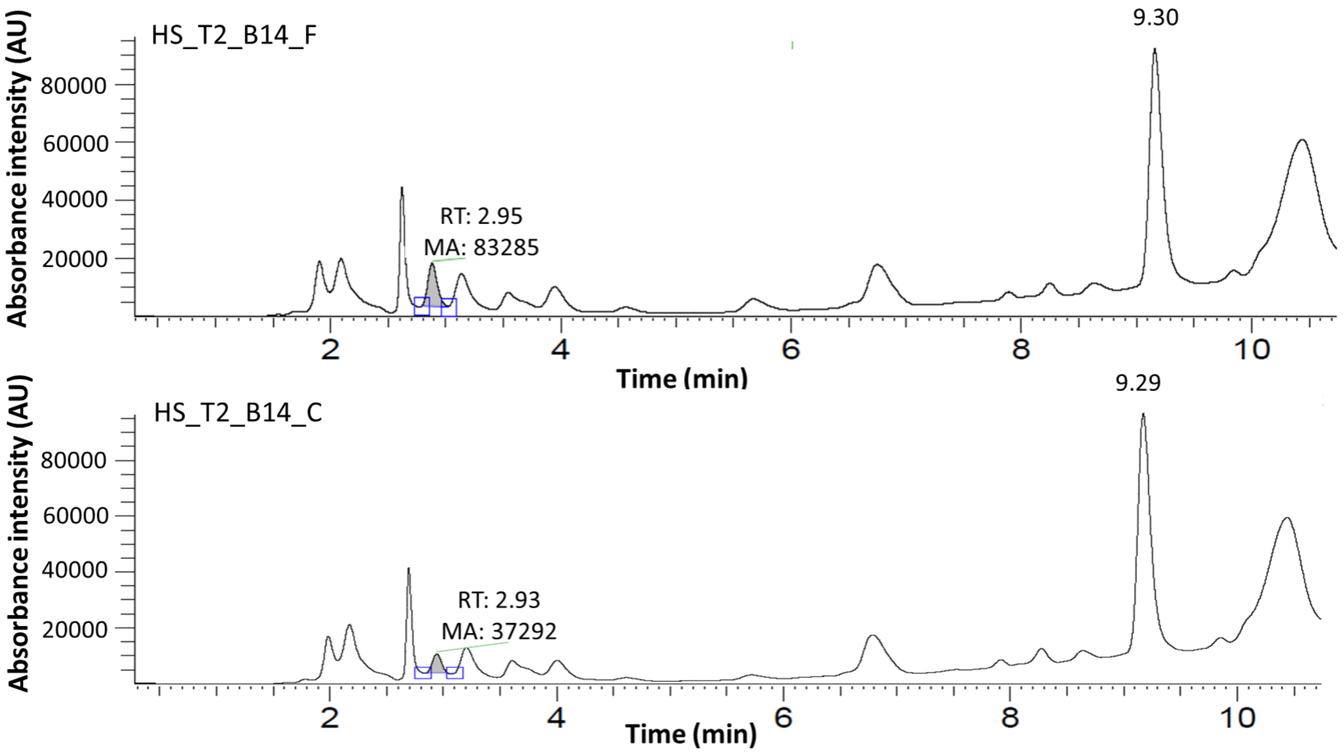


Figure 63: Chromatogram of *Hibiscus sabdariffa* calyx extracts obtained from HS_T2_B14 powders; RT - retention time, MA - molecule area, AU - arbitrary unit.

4.2.3.5. *Influence of powder reconstitution properties on anthocyanin release*

A strong negative correlation between dispersion rate in phase 1 and reconstitution time (R = -0.94) on one hand, and between the dispersion rate in phase 1 and anthocyanin release time (R = -0.86) on the other hand was observed (Table 8). This reveals a great influence of the dispersion step on the release of water-soluble compounds. A rapid dispersion of particle agglomerates results in a short release time of soluble molecules, especially anthocyanins. During the reconstitution, the dispersion step facilitates the extraction of biomolecules from hibiscus calyx powders by increasing the particle specific surface area and then the area of the solvent interface.

Table 8: Pearson correlation matrix of flowability, particle size, reconstitution, and anthocyanin extraction characteristics of *Hibiscus sabdariffa* calyx powders.

Variables	**Particle median diameter (D50)**	**Cohesion**	**Chroma**	**Anthocyanin concentration after 30 min reconstitution at 20 °C**	**Anthocyanin concentration after 30 min reconstitution at 50 °C**	**Swelling rate**	**Dispersion rate phase 1**	**Dispersion rate phase 2**	**Reconstitution time at 20 °C**	**Anthocyanin release time at 50 °C**
Particle median diameter (D50)	**1**	0.428	**-0.990**	**-0.903**	-0.733	0.581	**-0.950**	0.741	**0.995**	**0.964**
Cohesion	0.428	**1**	-0.483	-0.287	-0.215	0.146	-0.187	0.292	0.414	0.509
Chroma	**-0.990**	-0.483	**1**	**0.853**	0.696	-0.514	**0.915**	-0.718	**-0.991**	**-0.954**
Anthocyanin concentration after 30 min reconstitution at 20 °C	**-0.903**	-0.287	**0.853**	**1**	0.786	**-0.843**	**0.867**	**-0.882**	**-0.877**	**-0.949**
Anthocyanin concentration after 30 min reconstitution at 50 °C	-0.733	-0.215	0.696	0.786	**1**	-0.439	0.796	-0.473	-0.668	-0.745
Swelling rate	0.581	0.146	-0.514	**-0.843**	-0.439	**1**	-0.498	**0.931**	0.567	0.720
Dispersion phase 1	**-0.950**	-0.187	**0.915**	**0.867**	0.796	-0.498	**1**	-0.606	**-0.936**	**-0.864**
Dispersion phase 2	0.741	0.292	-0.718	**-0.882**	-0.473	**0.931**	-0.606	**1**	0.746	**0.857**
Reconstitution time at 20 °C	**0.995**	0.414	**-0.991**	**-0.877**	-0.668	0.567	**-0.936**	0.746	**1**	**0.950**
Anthocyanin release time at 50 °C	**0.964**	0.509	**-0.954**	**-0.949**	-0.745	0.720	**-0.864**	**0.857**	**0.950**	**1**

The current study permits to suggest a mechanism for the reconstitution of hibiscus calyx powders in water (Figure 64).

(1) **Wetting/sinking**. This step, not evaluated in this study, has certainly allowed the molecules accessible on the surface to quickly dissolve.

(2) **Molecular solubilization**. Water diffuses into the particle by entering through the pores. The water-soluble compounds, are transferred from the particle to the aqueous medium. For example, anthocyanin molecules are found in the form of flavylium ion derivative in aqueous medium. This exchange of matter results in molecular solubilization, conductivity increase, red-purple coloration of the solution, and discoloration of hibiscus particles.

(3) **Swelling**. At the same time, the particles filled with water begin to swell resulting in size increase and structure weakening, which become more sensitive to the shear induced by the stirring forces.

(4) **Dispersion**. It starts at the same time as the swelling phase. First, the water penetrates the narrow spaces between the aggregated particles and causes, under the complementary effect of stirring, the dissociation of particle aggregates. Secondly, the pressure exerted by the water inside of the particle results in the fragmentation of mother particles into soluble and insoluble daughter particles.

Once the reconstitution is finished, the soluble particles disappear and only the non-soluble ones remain.

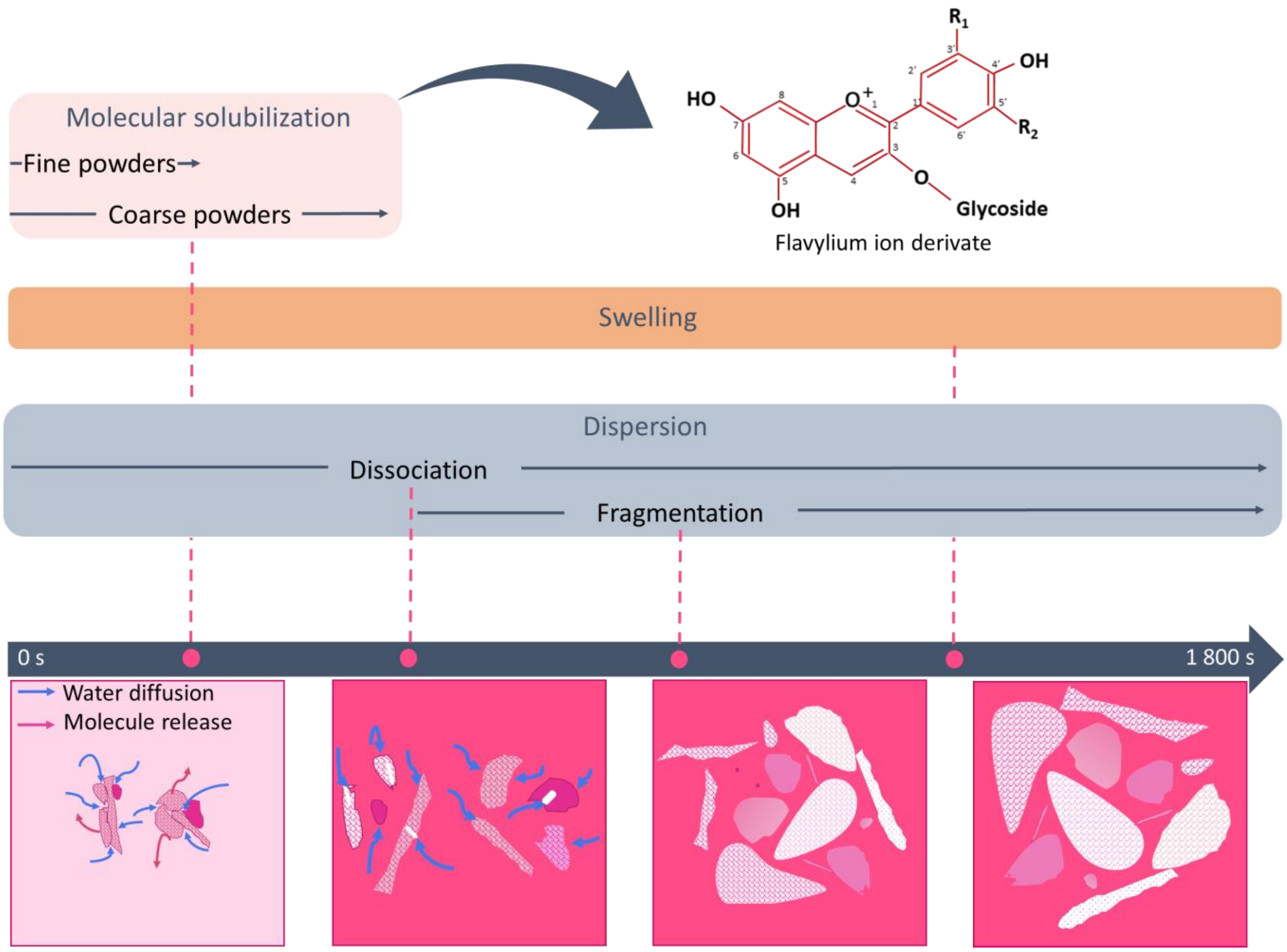


Figure 64: Reconstitution mechanism of *Hibiscus sabdariffa* calyx powders.

4.2.4. Conclusion

The grinding and fractionation by sieving allow producing powders with singular physicochemical properties. On the one hand, these properties including physical (particle size, heterogeneity), and the chemical composition (water content, fiber content) are related to the powder flow properties. The reduction in particle size and the high heterogeneity of the powders induced a great compressibility of powders so that they are sensitive to the application of stresses during processing or storage. In this sense, the powder compressibility should be a determining factor in the choice of the packaging type. The low cohesion of the hibiscus powder fractions favors a reduction of the risk of particle agglomeration and caking whatever their granulometric differences.

On the other hand, the elucidation of the proposed reconstitution mechanism was carried out by combining several methods. Firstly, scanning electron microscopy and morphogranulometry were used to follow the particle structure evolution. Secondly, methods were applied to characterize the reconstitution medium: measurements of coloration, conductivity, anthocyanin content, turbidity (insoluble material). Taken together, all of these results allowed establishing a common reconstitution mechanism. Three main individual steps were identified: quasi-instant solubilization of hydrophilic molecules, particle swelling and dispersion (dissociation + fragmentation) which took place simultaneously. These reconstitution steps are also inherent to the powder physicochemical properties (size, shape, porous structure, fiber content, interparticle forces), and the powder composition (anthocyanins, polyphenol, carbohydrate, fiber), and extrinsic factors such as the type of agitation, the agitation speed, and the solution temperature.

The reconstitution mechanism study allows evidencing that the combination of drying, grinding and sieving allow to obtain two fractions with individual application properties: fine powders with an almost instantaneous solubilization (30 s) in either hot water (50 °C) or cold water (20 °C) and coarse powder whose reconstitution requires an infusion time of 2 to 5 min. The advantage of performing the extraction at 20 °C rather than 50 °C (optimal extraction temperature for anthocyanins) is the reduction of the risk of degradation of heat sensitive biomolecules.

Fine powders can be presented as high added value products, as they are as rich in anthocyanins as coarse powders, and have the highest nutritional value (antioxidant activity), hence the interest to valorize them. However, the solubility of fine powders still have to be improved to obtain instant powder and avoid cloudy drink which is a criterion that negatively impacts the product acceptability. To overcome this aspectual issue, it is possible to reduce the particle size, the sieving time and/or to perform a sieving by horizontal vibrations. From an industrial point of view, it is also possible to slightly centrifuge the extracts of hibiscus powders or to let them rest in cold water and recover only the supernatant. The fractionation by sieving as well as the understanding of the reconstitution of the hibiscus calyx powders allow to properly valorize powders by defining their specific functionalities and uses.

The previous chapters have highlighted the importance to control the drying and grinding parameters, and also the contribution of the fractionation operation in the production process of hibiscus powders. These studies emphasize two critical points to be considered: the drying parameters (type of drying, time/temperature couple) and the heterogeneity of powders. The latter are therefore fundamental points for possible improvements in functional properties (flowability and reconstitution), and for obtaining ready-to-use, minimally processed, hibiscus powders that are in line with FAO and SDGs objectives (sensitization to plant consumption and biodiversity, limitation of food waste, the economy support of large-scale farming and family farming).

The third part of this PhD work deals with the above-mentioned points of improvement and prioritizes the nutritional quality based on the powder antioxidant activity, by evaluating the properties of the fractionated powders obtained by solar and controlled drying (by oven).

Introduction à la troisième partie des résultats

L'*Hibiscus sabdariffa* (hibiscus) fait partie de ces aliments riches en antioxydants davantage demandés par les consommateurs car étant particulièrement riches en anthocyanes. Etant donné les habitudes et tendances du marché actuel, un séchage solaire traditionnel et un séchage par étuvage (un séchage contrôlé à 55 °C / 24 h) des calices d'hibiscus, suivis d'un broyage et d'un fractionnement par tamisage ont été étudiés et comparés dans le but de produire des poudres d'hibiscus dites peu transformées, et facile à utiliser. Pour cela, les propriétés fonctionnelles, les propriétés physiques et chimiques ont été évaluées, notamment la teneur en anthocyanes et l'activité antioxydante qui ont été mesurées avant et après un stockage de 30 jours à 4 °C et 20 °C. La réduction en poudre fines (diamètre médian ≈ 70 µm) des calices issus du séchage contrôlé a permis d'améliorer l'extractibilité des anthocyanes et également d'obtenir des poudres présentant une reconstitution quasi-instantanée. Cette disponibilité des biomolécules, et l'évaluation des concentrations en anthocyanes a mis en exergue un effet positif du procédé de séchage contrôlé + broyage + tamisage : la préservation des molécules d'intérêt pendant la transformation en poudre. Il est important de noter que, quel que soit le procédé employé, l'activité antioxydante des poudres d'hibiscus reste quasiment stable durant leur stockage pendant un mois et donc que le procédé de production de la poudre impacte uniquement l'activité antioxydante initiale.

Par ailleurs, la compressibilité des poudres fines étant très importante en raison de la taille/forme des particules et de la proportion de particules fibreuses, il serait avantageux de considérer cette compressibilité pour le choix de l'emballage et des conditions de stockage des poudres dans un but d'éviter la prise en masse des poudres.

Ce travail a fait l'objet de la rédaction d'une troisième publication qui sera prochainement soumise : Improvement of the functional properties and antioxidant activity of *Hibiscus sabdariffa* powders by applying controlled drying and fractionation.

4.3. Improvement of the functional properties and antioxidant activity of *Hibiscus sabdariffa* powders by applying controlled drying and fractionation: Article n°3

4.3.1. Introduction

The antioxidant activity of a product is defined by its capacity to scavenge free radicals responsible for cellular oxidative stress, which causes degenerative pathologies. Among the prevention methods of disease risks studied, nutrition has been recognized as fundamental to improve the life quality (Prenesti et al., 2007). This includes the consumption of fruits and vegetables recommended by FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) and proven to improve the general well-being of consumers, because they are rich sources of polyphenols (FAO, 2018; FAO and OMS, 2004; Tomás-Barberan et al., 2000). These polyphenols, are potent antioxidant molecules (Mellor and Naumovski, 2016; Rhodes, 1996), and include flavonoids whose antioxidant activity changes with structural modification (Heim et al., 2002). These structural changes could occur during thermal food processing hence the need to apply minimal processing. Anthocyanins, a flavonoid group, are heat-sensitive biomolecules responsible for the red color of *Hibiscus sabdariffa* (hibiscus) calyxes, which also present good antioxidant activity. However, due to their high water content (80 - 90 %) (Cisse, 2010) hibiscus calyxes are perishable, therefore they are necessarily dried to be stabilized. The traditional method to reduce water content and water activity and then to extent the calyx shelf-life is solar-drying. Another drying objective is to inactivate enzymes e.g. anthocyanin-β-glucosidase that hydrolyze anthocyanins and could consequently reduce the product hue (Jurić et al., 2020; Todaro et al., 2008). Given the thermal instability of anthocyanins, and other phenolic components (protocatechuic and catechic acids) of hibiscus calyxes (Balzarini et al., 2018; Cheng et al., 2014; Tham et al., 2018), they could also degrade when they are subjected to high temperatures, or long duration of heat exposure (Sadilova et al., 2006; Seeram et al., 2001; Sinela, 2016). Studies reported a reduction of about 15.3 % phenols and 36.9 % anthocyanin in sun-dried calyxes (Cheng et al., 2014; Tham et al., 2018). In addition to the reduction in antioxidant activity induced by this loss in biomolecules, the results also showed a change in calyx coloration. Calyxes became more red and yellow, because of material browning as a result of Maillard reaction (Tham et al., 2018). In addition, after oxidation reaction in an oxygenated environment, the oxidized products would react with anthocyanins, producing colorless and brown molecules (Jackman et al., 1987). However, the calyx red coloration is a strong advantage from a marketing point of view, since the appearance is decisive in the purchase act of a product by the consumer. Considering the thermo-sensitivity of biomolecules, hot air drying at 50 °C for 8 h or 70 °C for 4 h showed better preservation of anthocyanins and at 70 °C / 4 h, 80 °C / 9 h for phenolic compounds (Nguyen and Chuyen, 2020; Sánchez-Feria et al., 2021). In the same conditions (hot air drying at temperature ≥ 50 °C) microorganisms development can be limited (Sánchez-Feria et al., 2021). Thus, controlled hot air drying proved better preservation of biomolecules than solar-drying, although low temperature and dehumidified air drying remains a better alternative (Tham et al., 2018). Controlled drying would result in minimally processed foods with a very good biomolecule preservation and availability.

To benefit from the advantages of hibiscus calyxes, soluble biomolecules (anthocyanin, phenols, sugar, acid...) extraction is usually performed in aqueous media to produce hot and cold drinks (Cisse, 2010). This extraction yield of anthocyanins is improved by reducing the calyx size, even more by producing powders. For example, for a average particle diameter of hibiscus calyx powders less than 150 µm, the anthocyanin extraction yield was increased by 65 to 125 % depending on the calyx/water ratio (Cisse, 2010). Reducing particle size improves anthocyanin extraction, extract coloration and reduces extraction time (Cisse, 2010; M’be et al., 2022). This observation is also supported by studies conducted on coffee powders, highlighting better caffeine extraction for fine powders (Baggenstoss et al., 2008).

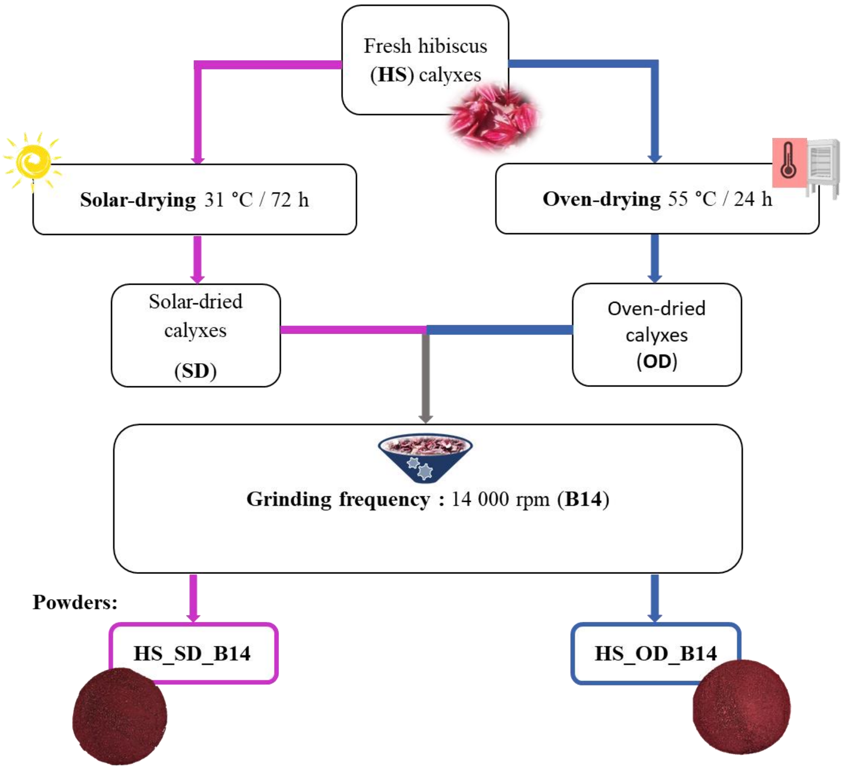
Considering all the above-mentioned advantages of drying and size reduction, controlled grinding preceded by drying is a good way to produce minimally processed hibiscus powders (i.e. powders keeping their maximum of their original inherent properties). Nevertheless, the produced powder polydispersity could induce poor flowability and high insolubility (M’be et al., 2022). Authors show that all these properties comprising insolubility, flowability, accessibility to biomolecules and antioxidant activity could be improved by applying particle size reduction and/or separation by fractionation (Deli et al., 2019a; M’be et al., 2022). The latter fractionation process, when associated with drying and grinding, facilitates the reproducibility of analyses by reducing the powder heterogeneity. Better yet, fractionation by sieving allowed a differential separation of molecules resulting in a repartition according to the powder particle size. For example, it was observed a good retention of carbohydrates, fibers, in coarse powders while fine powders were rich in phenols, with higher antioxidant activity (Deli et al., 2019a). Such a method allows to concentrate interesting molecules in a fraction that will be better valorized. This paper proposes a comparative study of the flowability properties, biomolecule preservation, and antioxidant activity of fractionated *Hibiscus sabdariffa* powders obtained from solar-drying and oven-drying. This pertinent comparison step is required to define the best method to produce minimally processed hibiscus powders presenting the best functional properties and to determine potential improvement points.

4.3.2. Materials and methods

Plant material (see section 3.1.1 – Second calyx harvest)

Powder production (see sections 3.2.2 and 3.2.3)

Harvested calyxes were separated in two batches, the first one was sun-dried (SD, 72 h) and the second one was oven-dried (OD) at 55 °C / 24 h. The drying time was adjusted according to the calyx water content and was stopped when reaching 12 g / 100 g on dry basis.



Manufacturing process of fresh *Hibiscus sabdariffa* calyx powders by controlled (oven-drying) or solar-drying and grinding **(figure 23, section 3.2.2)**.

These two calyx batches were ground (14 000 rpm) and fractionated by sieving to obtain fine (F) and coarse (C) powders from sun-dried (SD_F and SD_C) and oven-dried (OD_F and OD_C) processes.

Powder physicochemical characterization (see section 3.3)

- Granulometry and morphology
- Powder flowability
- Determination of water content and water activity
- Measurement of powder color

Powder extract analysis (see section 3.4)

- Determination of browning index
- Determination of anthocyanin content
- Determination of antioxidant activity

4.3.3. Results

4.3.3.1. Drying duration determination

The most commonly used drying method for hibiscus calyxes is sun-drying, since the sun is easily accessible in tropical countries. However, in a way to better manager the powder production chain, and for providing a minimally processed powder, oven-drying has been evaluated. This evaluation focuses on the simultaneous quantification of the anthocyanin content and the water loss of the calyxes during drying (Figure 65).

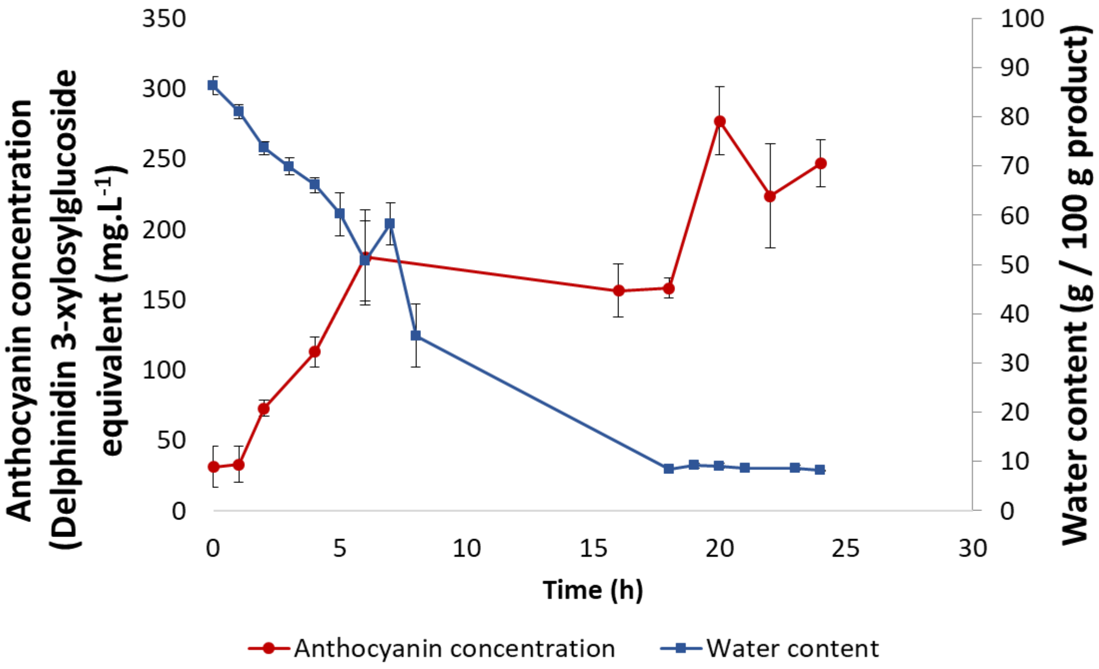


Figure 65: Kinetics of anthocyanin concentration and water loss during oven-drying of *Hibiscus sabdariffa* calyxes.

The decrease in water content and the increase in anthocyanin concentration occur simultaneously during the whole drying period (24 h). The reduction in water content induces a concentration of anthocyanin molecules in the hibiscus calyxes. After 18 h of drying, the water content becomes progressively stable, the remaining water is probably bound water. This stability is difficult to observe for the anthocyanin concentration due to the high variability of the last points of the curve. However, a decreasing trend in the anthocyanin concentration curve from 20 h of drying suggests a destruction of these compounds. This trend can also be explained by the variability of the samples and the sampling. Anthocyanin concentration upon the oven-drying shows a good preservation of the nutritional quality of calyxes dried at 55 °C for at least 20 h.

About sun-dried fresh calyxes, only initial and final water contents (90 g/100 g and 11.85 g/100 g water on dry basis, respectively) and anthocyanin concentrations (25 and 100 delphinidin 3-xyloglucoside equivalents mg.L^{-1}, respectively) have been evaluated for better time management.

4.3.3.2. Powder physicochemical properties

Water content and water activity. As desired, the dried hibiscus calyxes had similar water contents whatever the type of drying applied (≈ 12 g / 100 g product). The corresponding water activities were below 0.3, which enable a limited bacterial, yeast and mold growth, lipid oxidation, as well as enzymatic reactions such as enzymatic anthocyanin hydrolysis by anthocyanase (Jurić et al., 2020; Labuza, 1977; Todaro et al., 2008). Water activity of oven-dried calyxes was the lowest (0.25), ensuring them a better preservation during shelf-life than solar-dried calyxes.

After grinding, differences in physicochemical properties were observed between whole and sieved hibiscus powders. Powders from OD process still had the lowest water content and water activity (Table 9). Water content and water activity of all fine and coarse powders were similar to those of whole powders, except the water content of the OD_C (Oven-Dried and Coarse) powders. This shows an almost equal distribution of water molecules in powder, which could still slightly vary depending on the composition of each powder particle, and the particle size.

Table 9: Physicochemical properties of *Hibiscus sabdariffa* powders; OD - oven-drying, SD - sun-drying, W - whole powder, F - fine powder, C - coarse powder.

Powder sample	Water content (g / 100 g on wet basis)	Water activity (-)	D_{50} (µm)	Span (-)	Aspect ratio (-)	Elongation (-)	Sphericity (-)
OD_W	10.43 ± 0.38 ^{c}	0.31 ± 0.01 ^{bc}	201.67 ± 9.87 ^{c}	3.48 ± 0.13 ^{a}	0.62 ± 0.01 ^{ab}	0.30 ± 0.01 ^{b}	0.80 ± 0.01 ^{a}
SD_W	11.74 ± 0.22 ^{b}	0.36 ± 0.00 ^{a}	222.67 ± 2.08 ^{c}	2.94 ± 0.01 ^{b}	0.62 ± 0.01 ^{ab}	0.31 ± 0.01 ^{b}	0.81 ± 0.00 ^{a}
OD_F	10.63 ± 0.09 ^{c}	0.30 ± 0.01 ^{bc}	69.93 ± 0.55 ^{e}	3.00 ± 0.03 ^{b}	0.65 ± 0.00 ^{a}	0.37 ± 0.00 ^{a}	0.81 ± 0.00 ^{a}
SD_F	12.05 ± 0.35 ^{ab}	0.36 ± 0.00 ^{a}	94.93 ± 7.04 ^{d}	2.91 ± 0.39 ^{b}	0.63 ± 0.00 ^{ab}	0.36 ± 0.00 ^{a}	0.81 ± 0.00 ^{a}
OD_C	11.61 ± 0.22 ^{b}	0.33 ± 0.00 ^{b}	392.67 ± 7.02 ^{b}	1.35 ± 0.03 ^{c}	0.60 ± 0.01 ^{b}	0.26 ± 0.01 ^{c}	0.80 ± 0.01 ^{a}
SD_C	12.33 ± 0.02 ^{ab}	0.37 ± 0.02 ^{a}	447.67 ± 14.01 ^{a}	1.31 ± 0.06 ^{c}	0.61 ± 0.03 ^{b}	0.26 ± 0.01 ^{c}	0.80 ± 0.01 ^{a}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey’s HSD test ($n = 3$, $p \leq 0.05$).

4.3.3.3. Powder physical characterization

Particle size and shape. The whole powders whatever the drying method are characterized by similar average particle diameters (Table 9). The type of drying has no obvious effect on the particle size of these powders, due to the close water contents of hibiscus calyxes. Having said that, OD powders are more heterogeneous (highest span value) than SD powders. Differences attributed to the drying method was observed after the fractionation step: OD_F and OD_C powders had smaller average particle sizes than those from sun-drying (Table 9). This can be explained by the drying effects on product structure and texture, which varies according to the drying method and parameters (temperature, duration, drying rate, air velocity, radiation) and consequently impacts the grinding efficiency (Ramos et al., 2003; Seerangurayar et al., 2018). Indeed, during the drying process, thermal and humidity gradients lead to a decrease in the turgor pressure of plant cell walls and result in a modification of the structure by collapse of the plant. The turgor pressure is the internal cellular pressure of the tissues that maintains the shape, firmness, crispness of the plant tissues (Ramos et al., 2003; Seerangurayar et al., 2019). During drying, the structure can also be modified by rupture of the plant cell wall and membrane (Seerangurayar et al., 2019). For example, authors evidenced that the forced convective solar-drying which requires lower time of treatment (60 h) less affects the shrinkage, and cell wall rupture of dried dates than the solar-drying (100 h) (Seerangurayar et al., 2019). This structure change is often accompanied by a modification of the texture (rigidity, friability). By increasing the temperature (decreasing the drying time), the product becomes easy to break (Jafari et al., 2016), which would facilitate the grinding and therefore influence the powder particle size distribution, unlike sun-drying during which water lengthily diffuses through the cells because of the long drying time.

From a morphological point of view, no influence of the drying method is observed on powder sphericity, aspect ratio, and elongation (Table 9). The aspect ratio values were all low (0.6 - 0.65), meaning that the particles are all irregular shaped, and elongated. The powder fractions (fine (F) and coarse (C)) present aspect ratios identical to those of the whole powders with the exception of the oven-dried calyx powders. In fact, OD_F are characterized by higher aspect ratios than OD_C, showing the high irregularity of coarse powder particles and assumes a low elongation value. In agreement with the aspect ratios results, the elongation values of fine powders are the highest (Table 9), so the particles of these powders are less elongated than the coarse powder particles. The presence of elongated particles in coarse powders is due to hibiscus fiber richness. Whole powders are characterized by intermediate elongation values between fine and coarse powders, which is rather obvious since they consist of a mixture of fine and coarse powders.

4.3.3.4. Powder flowability

Compressibility values are presented in Table 10. Compressibility of coarse powders (OD_C and SD_C) are the lowest, followed by those of whole powders and finally compressibilities of fine powders, which are the highest. Powder compressibility values can be explained by the particle size and shape (irregularity and elongation) (Figure 66). Indeed, irregular-shaped and smaller particle tend to trap a lot of air (Irie et al., 2021; Salameh et al., 2016), leading to high interparticle porosity which results in high compression sensitivity of powders. Since the majority of powders have similar shape parameters, particle size is then the determining point in understanding the compressibility of these powders. In the same sense, due to its smallest particles size, OD_F powder compressibility is higher than that of SD_F powder.

Table 10: Flowability and color parameters of *Hibiscus sabdariffa* powders.

Sample	Compressibility at 15 kPa (kPa)	Cohesion (kPa)	Flow factor (-)	L* (-)	a* (-)	b* (-)	Chroma (-)	Color
OD_Calyx	-	-	-	39.31 ± 0.06 ^{c}	6.17 ± 0.04 ^{e}	-2.06 ± 0.07 ^{d}	6.51 ± 0.04 ^{e}	■
SD_Calyx	-	-	-	38.79 ± 0.21 ^{c}	5.97 ± 0.26 ^{e}	-2.35 ± 0.12 ^{e}	6.42 ± 0.21 ^{e}	■
OD_W	13.8 ± 0.47 ^{c}	0.66 ± 0.01 ^{a}	7.3 ± 0.15 ^{b}	39.31 ± 0.12 ^{c}	6.64 ± 0.1 ^{e}	-2.04 ± 0.04 ^{d}	6.95 ± 0.10 ^{e}	■
SD_W	13.13 ± 0.19 ^{c}	0.61 ± 0.13 ^{a}	8.1 ± 1.48 ^{b}	46.74 ± 0.03 ^{a}	19.32 ± 0.1 ^{b}	0.17 ± 0.01 ^{b}	19.32 ± 0.10 ^{b}	■
OD_F	21.65 ± 0.14 ^{a}	0.7 ± 0.02 ^{a}	6.34 ± 0.19 ^{b}	46.78 ± 0.26 ^{a}	23.20 ± 0.65 ^{a}	0.32 ± 0.04 ^{ab}	23.20 ± 0.65 ^{a}	■
SD_F	17.07 ± 0.75 ^{b}	0.57 ± 0.01 ^{a}	7.8 ± 0.07 ^{b}	47.31 ± 0.05 ^{a}	20.12 ± 0.02 ^{b}	0.39 ± 0.01 ^{a}	20.12 ± 0.02 ^{b}	■
OD_C	6.05 ± 0.13 ^{d}	0.34 ± 0.08 ^{b}	14.37 ± 2.49 ^{a}	44.23 ± 0.95 ^{b}	15.82 ± 1.48 ^{c}	-1.25 ± 0.05 ^{c}	15.87 ± 1.48 ^{c}	■
SD_C	5.59 ± 0.05 ^{d}	0.29 ± 0.08 ^{b}	16.77 ± 3.46 ^{a}	43.83 ± 0.01 ^{b}	12.8 ± 0.01 ^{d}	-1.22 ± 0.01 ^{c}	12.86 ± 0.01 ^{d}	■

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p \leq 0.05$).

The present compressibility and cohesion results (Table 10) follow the same trend: The most compressible fine powders (OD_F and SD_F) are more cohesive than the less compressible coarse powders. This higher sensitivity to cohesion of fine powders can be explained by the lower particle average diameter. In accordance with Gnagne et al. (2017), high powder sensitivity to compression are linked to high cohesion. In addition, the identical values of fine powder to whole powder cohesion confirm the great influence of particle smallness on cohesiveness. There was no impact of the drying method on cohesion may be due to the general similitude of granulometric (particle size, span) and shape parameters between oven-drying and sun-drying. This high cohesion of OD_F and SD_F powders is due to their higher interparticle forces than coarse powders, which is caused by their higher specific surface area, irregular and elongated particle shape. Indeed, these particle shape and size of fine powders induce more contact points between particles (Gnagne et al., 2017; Petit et al., 2017), increasing the interparticle forces and resulting in higher cohesion. This cohesion level is nevertheless low and implies a free-flowing behavior for OD_F and SD_F powders. Coarse powders that are three times less cohesive, have the best flowability, they flow easily. Therefore, these fine or coarse powders could easily be handled in controlled environments (humidity and temperature) considering the sensitivity to compression of fine powders. Such a powder (OD_F and SD_F) must be handled avoiding compression, to prevent possible particle agglomeration.

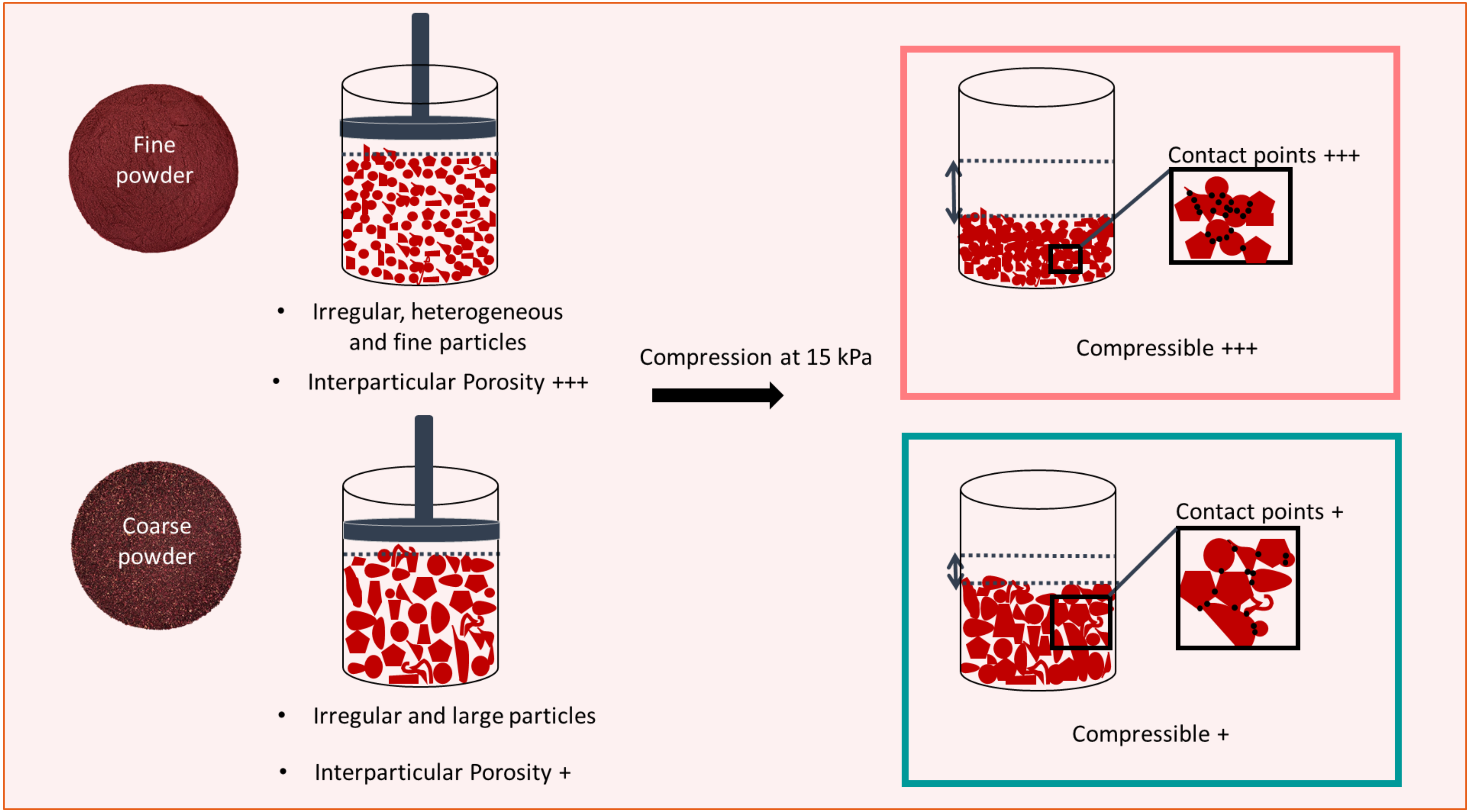


Figure 66: Sensitivity to compression of *Hibiscus sabdariffa* powders as a function of particle size and shape.

4.3.3.5. Color preservation and effect of browning molecules

Chroma, and the L*, a*, b* parameters (Table 10) were determined for dried calyxes and resulting powders. First of all, it can be observed that color parameters are clearly different between calyxes and powders which is explained by the impact of material structure and granulometry on L* a* b* measurements. For that reason, comparisons will only be performed between samples having almost the same structure. The color parameter values of hibiscus calyx surface from solar-drying are almost identical to those from oven-drying. However, the b*, a* parameters and the chroma of whole powder from oven-drying are lower than those obtained from solar-drying, whereas powders (fine and coarse) from oven-drying are mainly redder (high a*) and have higher chroma values compared to the powders obtained by solar-drying. This chroma value could reflect the presence of new molecules coming from undesired reactions.

The impact of brown molecules was evaluated by measuring the browning index (BI) of powders (Table 11). There was no significant difference between the BI of calyxes from oven-drying (BI = 0.36) and those from solar-drying (BI = 0.39), this may be due to their low specific surface area. After powder processing, only OD_W and SD_W BI values were different. The browning index of the OD_W powder was lower compared to that of the SD_W powder. This result in agreement with their chroma values, underlines a marked impact of solar-drying on product during transformation. The browning could be the result of the concomitant treatment by solar radiation, temperature and oxygen (Felicetti and Schrader, 2009; Nguyen and Chuyen, 2020). The difference in browning between OD_W and SD_W powders could be the result of the long duration and solar radiation exposure of sun-drying (72 h) compared to the oven-drying (24 h). Heat treatments cause the formation of brown molecules through the browning reaction, which in turn modifies the whole product coloration.

Table 11: Physicochemical properties of *Hibiscus sabdariffa* powders before and after a 30-days storage.

Powder sample	Insoluble material	Browning index	Initial antioxidant activity	Antioxidant activity after 30-days storage at 4 °C	Antioxidant activity after 30-days storage at 20 °C
	(%)	(-)	(µmol gallic acid / g dry matter)		
OD_W	50.47 ± 1.37 ^{a}	0.36 ± 0.03 ^{b}	27.21 ± 0.55 ^{ab}	21.49 ± 2.63 ^{bcde}	24.73 ± 2.62 ^{abc}
SD_W	43.51 ± 2.52 ^{b}	0.49 ± 0.08 ^{a}	14.55 ± 5.45 ^{cde}	14.48 ± 4.73 ^{cde}	11.03 ± 1.39 ^{e}
OD_F	39.29 ± 1.91 ^{b}	0.36 ± 0.02 ^{b}	33.58 ± 0.81 ^{a}	22.42 ± 0.49 ^{bcd}	22.13 ± 4.00 ^{bcd}
SD_F	42.67 ± 0.59 ^{b}	0.40 ± 0.03 ^{ab}	14.66 ± 5.52 ^{cde}	15.98 ± 2.17 ^{cde}	13.89 ± 7.38 ^{de}
OD_C	40.73 ± 2.11 ^{b}	0.35 ± 0.00 ^{b}	33.93 ± 0.83 ^{a}	21.73 ± 2.77 ^{bcde}	17.35 ± 0.38 ^{bcde}
SD_C	30.13 ± 2.44 ^{c}	0.38 ± 0.02 ^{b}	15.20 ± 4.60 ^{cde}	11.25 ± 1.95 ^{e}	13.21 ± 4.01 ^{de}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey’s HSD test ($n = 3$, $p \leq 0.05$).

4.3.3.6. *Biomolecule extractibility*

Kinetics of anthocyanin release presented in **Figure 67** show a remarkable difference between solar-dried (traditional drying) and oven-dried (controlled drying) powders. The anthocyanin concentrations of traditionally dried calyx powders are about 2 times lower than in oven-dried powders. The anthocyanin loss upon solar-drying is the most marked. Temperature accelerates all degradation reactions (Adams and Ongley, 2007; Palamidis and Markakis, 1975; Sinela, 2016) including anthocyanin degradation during drying. Increasing the temperature (with a pH range from 2 to 4) causes hydrolysis of the glycosidic bond, which leads to the loss of the anthocyanin glycosyl groups. Consequently, these anthocyanins become less stable as they are converted into their aglycone form (Adams, 1973; Sinela, 2016) which can be degraded by splitting the molecule ring (Sadilova et al., 2006; Seeram et al., 2001; Sinela, 2016) or transformed into chalcone molecules (Adams, 1973). This degradation is more noticeable for solar-drying powders because of the long exposure at variable temperatures (temperature depending on weather) contrary to the other powders coming from oven-drying. These results are in accordance with those of Nguyen and Chuyen 2020 working on Roselle tea. Indeed, solar-drying is almost not controlled, and dependent on weather: sunlight duration per day (9-10 h in this work), the night duration, air humidity, cloudy weather, are some of external conditions impacting the drying efficiency.

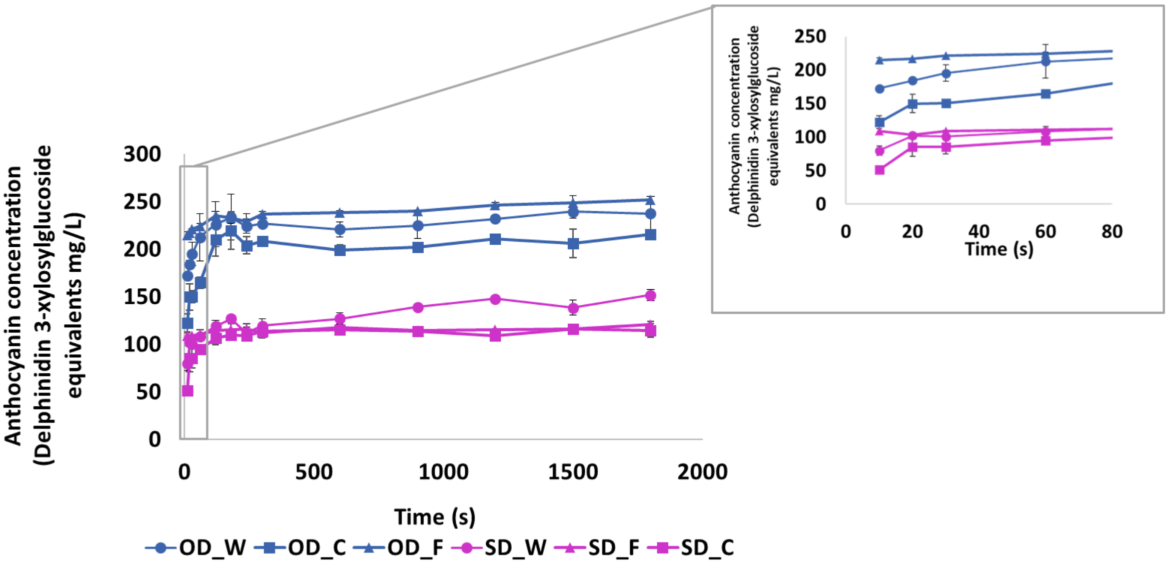


Figure 67: Anthocyanin release kinetics of *Hibiscus sabdariffa* powder in aqueous medium at 50 °C.

Furthermore, fractionated powders of solar-dried calyxes showed almost similar concentrations (100 - 150 delphinidin 3-xyloglucoside equivalents mg.L^{-1}) at 30 min extraction time but their anthocyanin kinetic releases were different. Indeed, the whole, fine, and coarse powders reached a plateau of anthocyanin concentration after about 180 s, 30 s, and 120 s of extraction respectively and are characterized by release times of 100 s, 10 s, 100 s (required time to release 90 % of anthocyanins). In the same way, fractionated powders of oven-dried calyxes are also characterized by similar final concentrations (200 - 250 delphinidin 3-xyloglucoside equivalents mg.L^{-1}), and differ in their anthocyanin release kinetics. The anthocyanin release from OD_F reach a plateau at 30 s and are extracted the fastest (release time = 23.33 ± 5.77 s) before the whole powders (release time = 70 ± 10 s) whose anthocyanin extraction is in turn faster than that of coarse powders (release time = 113.33 ± 11.55 s). Whatever the powder, anthocyanins in fine powders dissolve faster than whole and coarse powders due to their large specific surface area, which results in almost instantaneous anthocyanin extraction.

These results show that the capacities of fractionated powder to release anthocyanins in water are linked to the particle size, shape, and the chemical composition. Indeed, the smaller and more regular the particle shape, and the less elongated it is, the faster the release of anthocyanins. Therefore, fractionation is an excellent way to control the granulometry of grinding-induced powders and simultaneously improve the soluble molecule release, hence the interest to valorize powder fractions.

Additionally, the released anthocyanin stability can be maintained at 50 °C without any degradation until at least 30 min (Figure 67).

Temperature is an extrinsic factor that facilitates the molecular solubilization probably by the matrix weakening, and allows an optimal extraction of anthocyanins. The anthocyanin release time changes with temperature variation. Thus, for an extraction at room temperature (20 °C) (Figure 68), anthocyanin release time is longer and estimated to be 68 s and 75 s for SD_F and OD_F powders respectively. As expected, the time is longer for coarse powders with values of and 691 s and 600 s for SD_C and OD_C powders respectively. These values at 20 °C are 2 to 6 times longer than extraction at 50 °C but both temperatures are applicable during domestic or industrial preparations. The advantage of working at 20 °C is the ease to apply more than 30 min extraction or to do any preparation with a limited risk to the anthocyanin molecules because of their thermal sensitivity. Production of fine powder beverages at 20 °C simplifies and shortens the hibiscus cold drinks preparation, which are very popular. Traditionally soluble materials are extracted by immersing the dry hibiscus calyxes in boiling water or by maceration for 3 h at room temperature (Cisse, 2010), then the insoluble material is removed by filtering. A better alternative is proposed in this work, fine powders from oven-drying can be reconstituted at room temperature by a simple addition of powder in water and then cooled down before being consumed. There is no need to heat, boil or macerate and then to cool them down before cold storage.

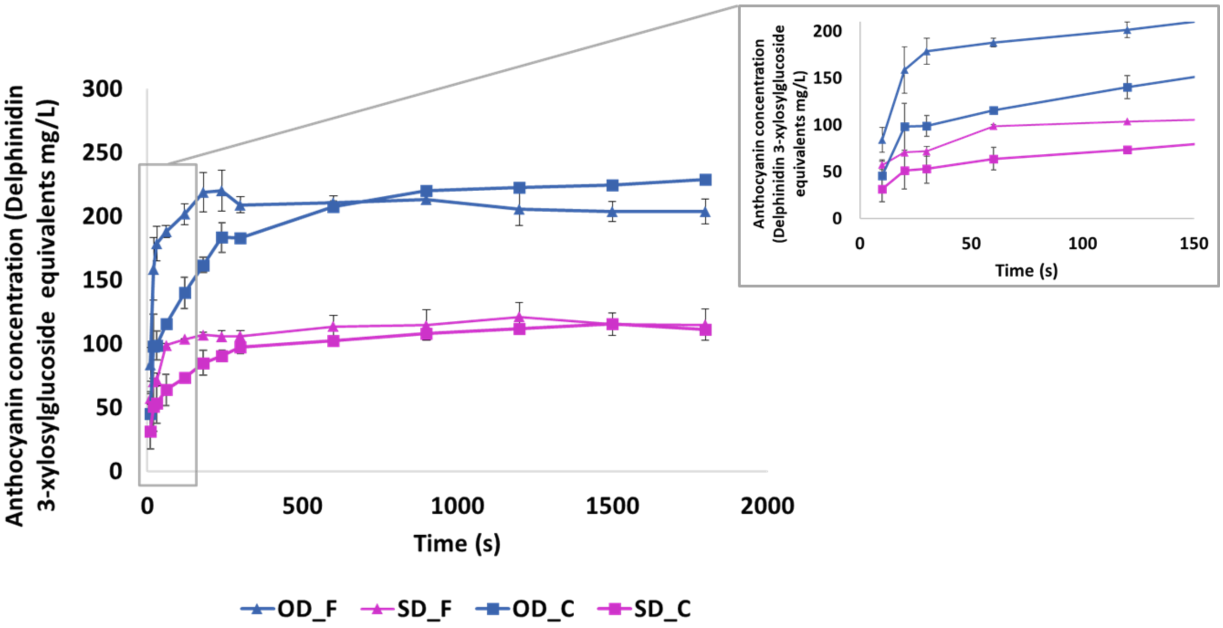


Figure 68: Anthocyanin release kinetics of *Hibiscus sabdariffa* powder in aqueous medium at 20 °C.

Overall, extraction temperature and release times are decisive characteristics to define the applications of the products, to explain the modes of use, and to optimize the production times in industry. In this sense, fine powders resulting from oven-drying, and the higher anthocyanin content could be used for direct applications, as an ingredient or as instant drink, and coarse powders for long preparation modes or infusions. Therefore, minimally processed vegetable powders with excellent biomolecules extractability is possibly achieved by applying a controlled drying (oven-drying) coupled to grinding, and fractionation.

4.3.3.7. Nutritional value of Hibiscus sabdariffa powder

The reduction in the antioxidant activity results from the anthocyanin degradation as well as a loss of polyphenols, phenolic acids (caffeoyl hydroxycitric acid, chlorogenic acid) upon the drying treatment (Mar et al., 2020; M. J. P. Monteiro et al., 2019; Prenesti et al., 2007). This antioxidant activity is the most affected when applying solar-drying because of the greatest biomolecule loss it induces. In addition, the antioxidant activity of all powders was almost the same after a 30-days storage at 4 °C and 20 °C, except for OD_F and OD_C powders. Without considering these two powders, there is no harmful impact of the time-temperature couple applied nor of the air humidity after 30 days of storage. The highest antioxidant activity loss of OD_F and OD_C could be explained by the following hypothesis: (i) a significant structure change induced by drying/grinding/sieving increase the biomolecule exposure to surface; (ii) for powders coming from solar-drying (SD_F, SD_C, SD_W), a significant amount of biomolecules are affected by the unit operations and those that have not been damaged during the process are no more exposed to the environment; (iii) the analysis method was not the most convenient to discriminate the powders. In this sense, a confrontation of the applied method with PAOT liquid technology could be a good way to understand the powder antioxidant activities. In addition, the OD_W antioxidant activity did surprisingly not change significantly during the storage. The powder heterogeneity would play a protector effect on the biomolecules. That said, OD_F and OD_C powder fractions have the highest antioxidant capacities, this allows obtaining high nutritional value fractions that are all valuable.

It is worth noting that these two powders (OD_F and OD_C) are considered poorly soluble (40 % insoluble material) because of the fiber richness (Table 11). Consequently, beverages prepared from these powders are cloudy and could hardly attract the consumer.

4.3.3.8. Principal component analysis (PCA)

The PCA result is presented in **Figure 69**. The F-axes (factors) are linear combinations of the variables (all properties studied: particle size and shape, powder flowability, anthocyanin release time, anthocyanin content, antioxidant activity…). The selected F1 and F2 axes represent 81.80 % of the variability of the information, so the representation on these two axes is of good quality. The correlation circle represented in **Figure 69**-a, corresponds to a projection of the variables onto a two-dimensional plane constituted by the two factorial axes F1 and F2. Based on the correlation, F1 axis is mainly related to macroscopic properties (shape and size distribution parameters, flowability properties). F2 axis expresses the molecular properties of powders (water content, water activity, color, anthocyanin content, antioxidant activity). The correlation circle shows that elongation, aspect ratio, and span are positively correlated with cohesion and compressibility, but negatively correlated with flow factor and anthocyanin release time. The particle size is positively correlated to flow factor, anthocyanin release time, but negatively correlated to span, elongation, aspect ratio, compressibility, and cohesion. This shows that compressibility and cohesion of powders increase with shape irregularity, particle size reduction, and powder heterogeneity, and this consequently reduces the powder flowability. Powders presenting a good flowability, and whose storage and handling conditions are controlled are less likely to agglomerate and cake. This avoids product returns by consumers, or losses and the efficiency reduction of the production process in industry. However, if large particles favor a good flowability, it is necessary to decrease the particle size to accelerate the anthocyanin release time, and produce instantaneous powders. A compromise is therefore necessary between anthocyanin release kinetics and powder flowability (powder handling), which can be achieved by improving the particle shape and the granulometric heterogeneities, for example.

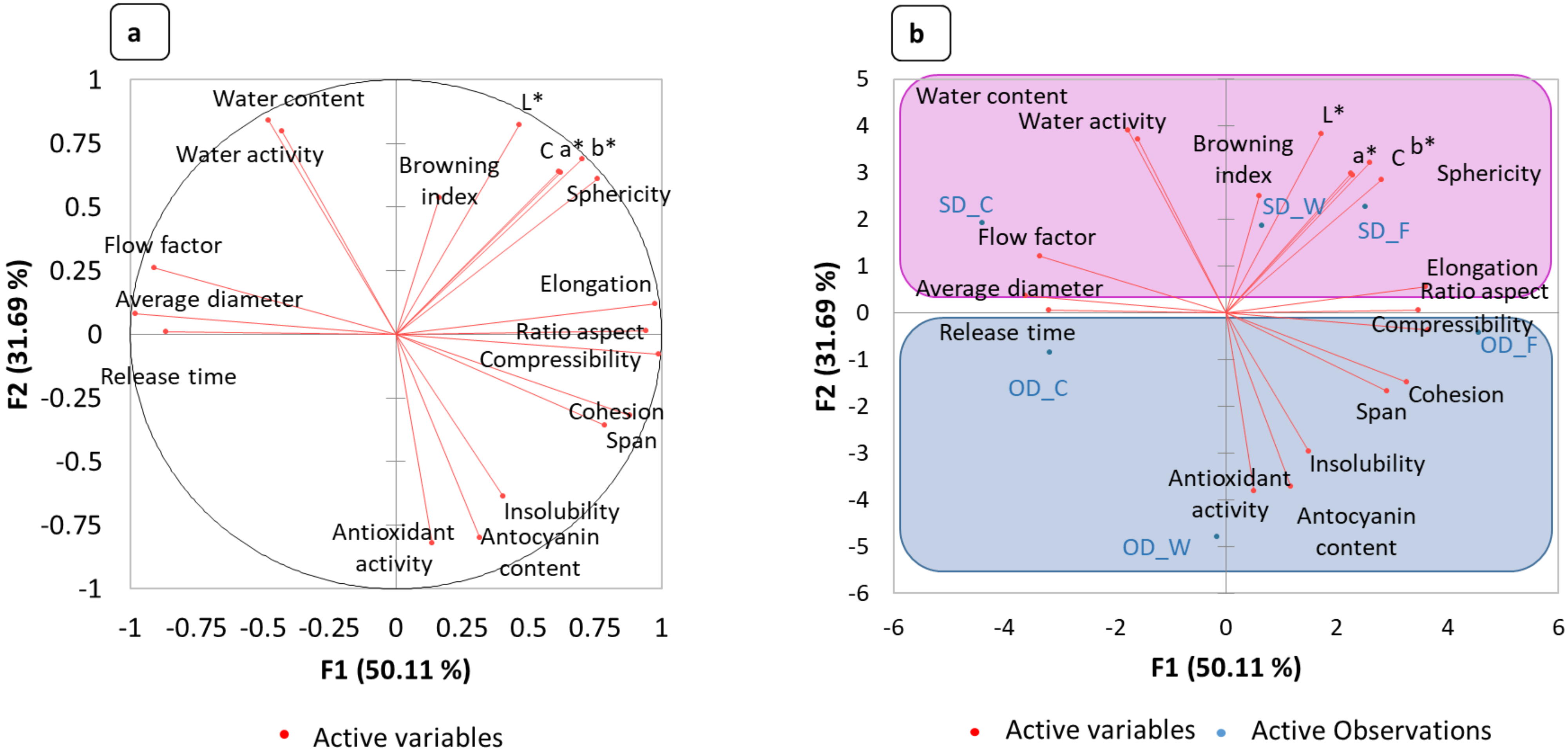


Figure 69: Principal component analysis of *Hibiscus sabdariffa* powder properties obtained from oven-drying (OD) or sun-drying (SD), a - correlation circle; b - representation of powders with the properties.

Moreover, the strong positive correlation between anthocyanin content and antioxidant activity (Figure 69-a) shows the important contribution of this molecule to the powder nutritional value in addition to its coloration. Due to their highest antioxidant activity, the OD_F and OD_C powders can also be applied as a food supplement to improve antioxidant activity of fermented milks and obtain a new functional foods (Su et al., 2020), or for dairy products, and different beverages (Echegaray et al., 2022). Hibiscus extract can also be employed for preventing oral diseases thanks to its antimicrobial activity (Sulistyani et al., 2016).

The powder distribution relies on Figure 69-b. According to the F1 axis, fine powders are grouped on the right side and coarse powders on the left side. This axis is thus linked to the fractionation process while the samples on the F2 axis are distributed according to the drying method: sun-dried powders are on top and oven-dried powders at the bottom. The sun-dried powder group is characterized by high water content, large and elongated particles, long release time, low anthocyanin content and low antioxidant activity unlike oven-dried powders. This second group of oven-dried powders is nonetheless compressible, with irregular and heterogeneous particles. These powders include fine powders (OD_F) that are full of active biomolecules and present an almost instantaneous reconstitution time as they require a short anthocyanin release time.

The PCA results confirm the impact of the drying method on the integrity of the biomolecules and on physical properties. All of this, in addition to grinding-sieving process improve the powder flowability and the biomolecules extractability.

4.3.4. Conclusion

Controlled (oven-drying) and traditional (solar-drying) drying processes could favor a good microbial quality (by reducing a_w), but only oven-drying ensures good nutritional quality by preserving biomolecules (e.g. anthocyanins) and antioxidant activity, although browning reactions remain a limiting factor. The choice of controlled drying parameters must imply the plant biomolecules sensitivity to heat and structural deformation. The latter could affect the final product physical and functional properties. In this work, oven-drying associated with a fine grinding of vegetables and a granulometric separation of powder particles enable the improvement of the powder flowability and extraction properties. This consequently maximizes the biomolecules release from powder particles into the solvent to benefit from the hibiscus health potentials. However, the drying-grinding-sieving processing method was not sufficient to get fully soluble fine powders, which is still a critical point to be improved for the product acceptability by the consumer and to be classified as instantaneous powders.

Chapitre 5 : Conclusions générales et perspectives

La transformation des calices d'*Hibiscus sabdariffa* (hibiscus) en poudre a avant tout l'avantage d'être une excellente méthode de conservation d'un produit initialement très périssable, et saisonnier. D'une part, l'importance de l'étape de réduction en poudre repose essentiellement, sur l'optimisation des coûts logistiques liés au stockage et au transport, facilitant ainsi les échanges commerciaux entre zones (ville et pays) productrices et non productrices. D'autre part, ce procédé assure une meilleure valorisation nutritionnelle des calices et améliore leurs fonctionnalités. La production de la poudre d'hibiscus par séchage contrôlé associé au broyage est une méthode qui permet d'obtenir des produits très peu transformés sans recourir à une étape préalable d'extraction de composés solubles qui pourrait entrainer une dégradation des biomolécules. Le choix de ce procédé repose par conséquent sur l'obtention de bonnes propriétés fonctionnelles des poudres.

La mise en place et l'évaluation de la réduction des calices d'hibiscus en poudres par séchage suivi du broyage a permis de mettre en évidence l'importance de la maitrise des opérations unitaires pour produire des poudres présentant de bonnes propriétés fonctionnelles notamment l'aptitude à l'écoulement, l'aptitude à la conservation, et l'aptitude à la reconstitution. L'écoulement de la poudre dépend des interactions interparticulaires ; si elles sont élevées, la poudre sera considérée comme cohésive et sera sensible à la prise en masse (mottage des poudres). L'évaluation de la bonne aptitude à la conservation a permis de mettre en exergue trois paramètres importants dont une faible activité de l'eau, une préservation des biomolécules et de leur activité antioxydante, et une faible voire une absence de produits issus du brunissement non-enzymatique. L'aptitude à la reconstitution des poudres d'hibiscus est fondée sur la solubilisation des biomolécules et est limitée par la présence de particules insolubles (particules fibreuses des végétaux).

La démarche expérimentale et les conclusions liées aux résultats ont été présentées sur la Figure 70. D'un point de vue statistique, l'influence prédominante de l'opération de séchage sur les propriétés physicochimiques et fonctionnelles des poudres initialement produites a permis d'orienter les travaux vers une méthode de séchage contrôlable et préservatrice de la qualité nutritionnelle des poudres, et qui a permis en plus, d'améliorer l'aptitude à l'écoulement des poudres.

En complément, l'analyse statistique a permis de souligner le fort lien entre les propriétés fonctionnelles et propriétés physicochimiques des poudres (Figure 70) dont la teneur en eau, la proportion de particules fibreuses, la granulométrie, la morphologie des particules, l'hétérogénéité sont les plus pertinentes. Cette hétérogénéité des poudres est induite par le broyage de l'hibiscus, en raison de la nature cellulosique des matériaux végétaux et peut être réduite par application d'un fractionnement par tamisage. Cette opération unitaire complémentaire favorise une séparation granulométrique de la poudre, et permet de produire en un procédé unique, plusieurs fractions de poudre (fines et grossières) caractérisées par des propriétés fonctionnelles singulières : une solubilisation quasi instantanée, une insolubilité réduite des particules, un important potentiel antioxydant des poudres fines contrairement aux poudres grossières moins riches en antioxydants et dont les propriétés physicochimiques sont affiliées à celles des infusions (extraction lente de molécules, insolubilité élevée).

Ces propriétés singulières constituent le socle de la valorisation des poudres d'hibiscus à travers des modes d'utilisation convenablement définis : les boissons instantanées et colorants pour les poudres fines, les infusions pour les poudres grossières.

Cette stratégie applicable à d'autres végétaux permet de valoriser l'ensemble de la matrice alimentaire (le calice entier), et permet par conséquent de limiter les pertes alimentaires en réduisant la production de déchets. Finalement, des poudres d'hibiscus prêtes à l'emploi, peu transformées ont pu être produites en répondant aux objectifs de la FAO et des ODD à travers la sensibilisation à : la consommation des végétaux, la biodiversité, la limitation du gaspillage alimentaire, le soutien économique de l'agriculture à grande échelle et de l'agriculture familiale.

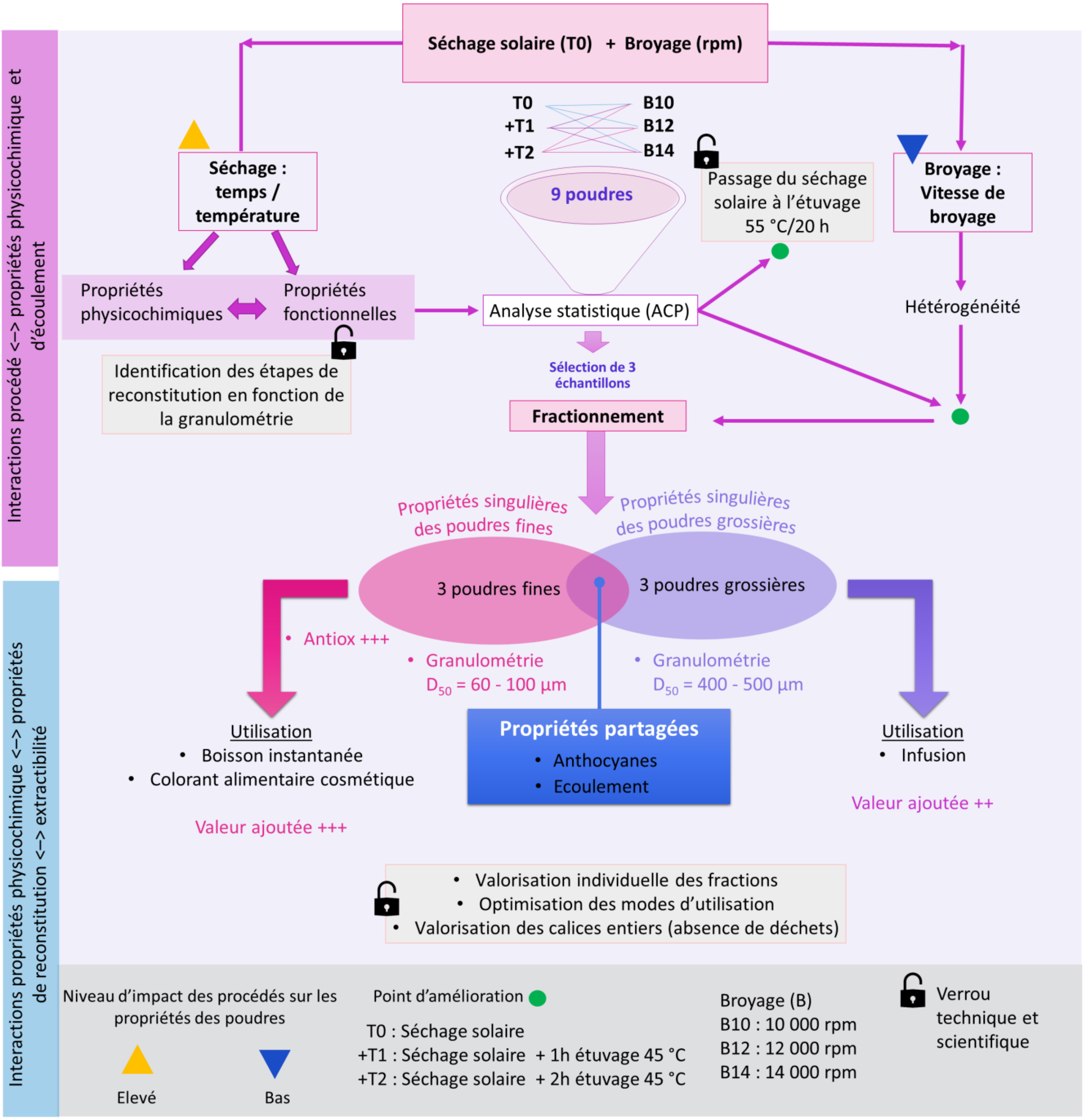


Figure 70 : Schéma récapitulatif de la mise en place du procédé de production de la poudre d'*Hibiscus sabdariffa*, l'impact sur les propriétés physicochimiques et fonctionnelles des poudres, et la valorisation des produits obtenus.

Des perspectives, pouvant servir à améliorer des travaux similaires ont découlé de ce projet. D'un point de vue expérimental, (1) la poursuite du séchage contrôlé au-delà de 24 h permettrait d'identifier le seuil de dégradation des anthocyanes ; (2) l'application d'un tamisage horizontal serait une alternative pour réduire la proportion de particules fibreuses des poudres ; (3) l'étude d'un vieillissement (réel et accéléré) permettrait de cibler les causes de dégradation des biomolécules et prévoir des méthodes palliatives de stabilisation telle que l'encapsulation des poudres ; (4) enfin la réalisation de tests d'acceptabilité du produit permettrait d'obtenir les données complètes nécessaires à la commercialisation de la poudre d'hibiscus.

(5) Pour une meilleure compréhension et maitrise des propriétés fonctionnelles des poudres d'hibiscus (aptitude à l'écoulement et à la reconstitution) ou des poudres végétales d'une manière générale, il serait pertinent d'évaluer les propriétés physicochimiques de la surface des particules. En effet, les surfaces sont les structures par lesquelles les particules interagissent entre elles. Il conviendrait donc d'analyser en complément, les propriétés physicochimiques de surface des particules par microscopie à force atomique (AFM) avec notamment des mesures de topographie et de nanomécanique. Par exemple, des surfaces irrégulières (forte rugosité) et la présence de lipides en surface favoriseront l'agglomération des particules. L'aptitude à la reconstitution est également limitée par la présence de lipides à la surface des particules.

(6) Par ailleurs, une étude sur d'autres matériaux végétaux servirait à confronter les observations réalisées sur l'hibiscus à des matrices présentant des structures similaires.

(7) D'un point de vue, environnemental et économique, il serait également convenable de mettre en place un protocole de production de poudre (séchage et broyage) respectueux de l'environnement et plus économique, en utilisant une énergie renouvelable largement disponible dans les pays producteurs tout en travaillant en conditions de séchage et broyage contrôlées pour avoir un produit conforme et constant.

(8) Enfin, d'un point de vue sociétal, il conviendrait d'échanger les connaissances avec les pays producteurs pour une meilleure valorisation des produits agricoles. La Côte d'Ivoire et la France par exemple, sont membres signataires du projet Nagoya qui réglemente les échanges de ressources naturelles et connaissances associées entre ces pays.

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Annexes

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Relationship between drying and grinding parameters and physicochemical properties of *Hibiscus sabdariffa* calyx powders

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ABSTRACT

To set up a stabilization process preserving the beneficial properties of *Hibiscus sabdariffa* calyxes, known as coupled drying and grinding, an option for powdering is proposed. The impacts of these unitary operations on powder physicochemical properties and flowability were studied. Calyxes were sun-dried and/or oven-dried for 1 or 2 h then ground at 10,000, 12,000, or 14,000 rpm. Upon increase in drying duration and/or grinding frequency, more heterogeneous and irregularly shaped particles of smaller-sized, low fiber powder was obtained which in turn affected the flow properties. Powders are less cohesive, therefore, they are less sensitive to compression and shear. This results in a good flowability. The red color intensity of powder solutions related to the anthocyanin content was raised when decreasing the drying duration and increasing the grinding frequency. However, to improve the solubility of these powder solutions, high drying duration and grinding frequency was required. High drying duration and grinding frequency may be ideal to achieve an easy-flowing powder, a long shelf life, and an appealing red-colored product for consumers.

KEYWORDS

Hibiscus sabdariffa; powder; drying; grinding; flowability; color intensity

1. Introduction

Hibiscus sabdariffa is an annual herbaceous plant of the Malvaceae family cultivated in many tropical and subtropical regions (Chumsri, Sirichote, and Itharat 2008; Cisse 2010; Ramírez-Rodrigues et al. 2011; Da-Costa-Rocha et al. 2014; Sinela 2016; Ahmed, Satti, and Eltahir 2019). The leaves and calyxes of this plant are all edible, and stems are employed for cattle feeding or textile. There are two types of calyxes of *Hibiscus sabdariffa,* – red and white ones – with a similar chemical composition (Ahmed, Satti, and Eltahir 2019). The red calyxes are particularly prized in the producer countries and also in foreign countries such as the United States of America and Germany (Plotto et al. 2004; Monteiro et al. 2019). The red color is the first appealing criteria of calyxes of *Hibiscus sabdariffa* followed by its sour-fruity taste and its high content in antioxidants. The *Hibiscus sabdariffa* calyxes are a rich source of water (90% water content), proteins, fibers, and carbohydrates (Cisse 2010). These calyxes are also particularly rich in mineral elements (K, Ca, Mg, Fe, Mn, Zn), organic acids (succinic, oxalic, malic, citric, stearic, tartaric, and ascorbic acids), and anthocyanins (Cisse 2010; Da-Costa-Rocha et al. 2014; Ahmed, Satti, and Eltahir 2019). Anthocyanins are responsible for the red color of *Hibiscus sabdariffa* calyxes and play an important role as an antioxidant.

This calyx composition, especially its high anthocyanin content, makes the *Hibiscus sabdariffa* a very coveted plant in several fields of activity such as cosmetics, pharmacy, medicine, and the food industry. In medicine, *Hibiscus sabdariffa* calyxes are used for their diuretic, febrifugal, anthelminthic, antimicrobial, hypotensive, and hypocholesterolemic activities, and for its ability to stimulate intestinal peristalsis (Johansen et al. 2005; Lee et al. 2009; Fullerton et al. 2011; Peng et al. 2011; Pérez-Torres et al. 2013; Da-Costa-Rocha et al. 2014; Paim et al. 2017; Seck et al. 2018). Moreover, *Hibiscus sabdariffa* calyxes are used in the food industry mainly to produce fresh beverages (fermented drinks, wine, etc.) and hot beverages like infusions. Cocktails, sirups, fruit salads, and pastry foods are also prepared from *Hibiscus sabdariffa* calyxes. In addition, delicious jellies, jams, and ice creams are manufactured and most appreciated by many populations all over the world (Plotto et al. 2004; Cisse 2010). For all these applications, hibiscus calyxes are either employed in fresh form or stabilized to facilitate transport and ensure good quality upon reception. The basic stabilization process consists of sun drying, which allows the reduction of moisture content and the prevention of microbial contamination (Labuza 1977). As a result, it extends the shelf life and controls the seasonality issue. Aqueous extracts of *Hibiscus sabdariffa* calyxes may also be prepared before being freeze or spray-dried. Spray-drying is the most applied process to obtain powders (Djaeni et al. 2018). However, the quality of *Hibiscus sabdariffa* calyx powders is altered during the stabilization process because of the degradation of anthocyanins. High extraction temperature and/or duration lead to higher extraction yields of dry matter but favor the loss of anthocyanins by thermal alteration. The thermal degradation

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of anthocyanins occurs by the rupture of these biomolecules, leading to the change in color of final products and a reduction of the antioxidant activity (Tsai et al. 2002; Chumsri, Sirichote, and Itharat 2008; Osman and Endut 2009; Cisse 2010; Idham, Muhamad, and Sarmidi 2012; Eroğlu, Tontul, and Topuz 2018).

To propose another powdering method, the current work focuses on the assessment of the influence of drying, which is achieved by sun-drying followed by oven drying, coupled with grinding on the functional properties (flowability, preservability) of *Hibiscus sabdariffa* calyx powders. To better understand the link between processing conditions and powder functionalities, their physicochemical properties (water content, water activity, particle size and shape, red color intensity, insolubility) were also determined.

2. Materials and methods

2.1. Plant material and sun drying

Hibiscus sabdariffa flowers from Ganaoni (9°17′5.935″N 6°19′22.262″W, Bagoue region), northern Ivory Coast, were collected in January 2019 and shelled to remove seeds and stems. Foreign objects were removed, then the calyxes were exposed directly to the sun (at about 37 °C) for at least 7 days until reaching a water content of 18.50 g/100 g on a wet basis. Sun-dried *Hibiscus sabdariffa* calyxes were finally packed in sealed bags and stored at 4 °C.

2.2. Oven drying and grinding

The global powder production process is presented in Figure 1.

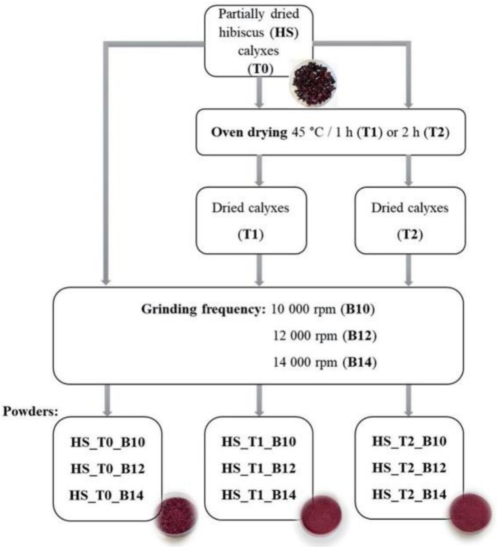

Figure 1. Process flow diagram of *Hibiscus sabdariffa* calyx powders.

2.2.1. Oven drying

Three batches of 900 g sun-dried calyxes were weighed and sorted. One of these batches was considered as the reference (T0) and was not subjected to oven drying. Two batches were dried in an oven (Memmert, ULM 400, Schwabach, Germany) at 45 °C for 1h (T1) and 2 h (T2) to further reduce the water content. The oven was preheated before introducing a perforated tray (1 cm holes) covered by a 1.5 cm thick calyx layer. The air velocity was assumed to be constant. After the drying process, the dried calyxes were preserved in a desiccator before grinding.

2.2.2. Grinding

First, calyxes were pre-ground in a blender (Moulinex, DPA1, Lourdes, France) for 5 s and then ground in an Ultra Centrifugal Mill ZM 200 (Haan, Germany) supplied with a 24-tooth rotor of 99 mm diameter, a stator consisting of a stainless steel ring sieve composed of 1 mm diameter trapezoid holes, and a < 1 cm feed hopper. Each batch of 900 g calyxes was divided into 3 batches of 300 g which were milled respectively at 10,000, 12,000, and 14,000 rpm (corresponding samples were respectively named B10, B12, and B14). Temperatures of all grinding-induced powders were measured to check that powders were not significantly heated. The powders were packed in sealed bags and stored at 4 °C, sheltered from light until analysis.

2.3. Powder physicochemical characterization

2.3.1. Water content and water activity

The water content was determined by the loss of water mass of 2.5 g powder for at least 36 h at 103 °C until a constant mass was obtained following ISO 21807: 2004 (AFNOR 2004). Water activity was determined **by** using a HygroPalm portable water activity meter (ART NO:HC2-AW, ROTRONIC, France) at 20 °C. More precisely, a 15 g sample was introduced into a polypropylene cup that was deposited in the sealed enclosure of the apparatus. The free water moistens or dries the air inside the enclosure until reaching equilibrium.

2.3.2. Color measurements

Powder color was determined using a CR-400 chromameter (Konica Minolta). For each sample, 10 g of powder was poured into a petri dish, and color measurement was performed according to the CIE L*, a*, b*color space. The lightness (L*) ranges from 0 (black) to 100 (white); the red-green balance (a*), from −100 (green) to 100 (red); and the yellow-blue balance (b*), varying from −100 (blue) to 100 (yellow). From these parameters, other colorimetric parameters can be calculated including the chroma (C*) and the hue angle (H*) related to saturation and tint, which are defined in Equations 1 and 2, respectively:

$$C* = \sqrt{(a*^2 + b*^2)} \tag{1}$$

$$H* \ = \ \arctan\left(b^*/a^*\right) \tag{2}$$

2.3.3. Particle size distribution

The Mastersizer 3000 equipped with the Aero S dry dispersion unit (Malvern Instrument, United Kingdom) was used to measure particle size by laser diffraction at 633 nm. About 5 g **of** powder was poured into the feed hopper and conveyed in the system using 30% vibration. The measurements were performed three times at a dispersing pressure of 1.5 bar, a hopper gap of 4 mm, an obscuration comprised between 0.5 and 1.5%, and a background calibration of 10 sec. The size estimator was the mean diameter (µm) in volume. Dispersion of particle sizes can be evaluated by measuring the span, which normalizes the width of the distribution relatively to the median value according to Equation 3:

$$\text{Span} \ = \ (d_{90} - \ d_{10})/d_{50} \tag{3}$$

Where:

- d10: 10% of the sample particles are smaller than this diameter,
- d50: 50% of the sample particles are smaller than this diameter,
- d90: 90% of the sample particles are smaller than this diameter.

2.3.4. Particle morphology

Particle morphology was examined using an optical microscope Olympus BH-2 (Japan) equipped with a 40 × magnification objective.

The dynamic image analysis system QICPIC (OASIS/L dry dispersing system, Sympatec GmbH, Clausthal–Zellerfeld, Germany) was used for particle size and shape characterization of the model blends in the dry dispersion mode. A dispersion pressure of 0.5 bar was applied. For each measurement ($n = 3$), approximately 0.5 g of powder was used. The images of investigated particles were captured by the camera and evaluated with the PAQXOS software. The perimeter of a circle of equal projection area (P_{EQPC}) (perimeter of a circle that has the same area as the projection area of the particle) and the shape characteristics (including elongation, aspect ratio, and sphericity) were determined.

Elongation (El) (0 <El <1) is a useful descriptive shape parameter for fibers, defined in Equation 4.

$$\text{El} \ = \ \text{fiber thickness } (\mu\text{m})/\text{fiber length } (\mu\text{m}) \tag{4}$$

The Aspect Ratio (AR) ($0 < \text{AR} \leq 1$) indicates the elongation and the level of irregularity of particles and is defined in equation (5).

$$\text{AR} \ = \ d_{\text{eret min}}(\mu\text{m})/d_{\text{Feret max}}(\mu\text{m}) \tag{5}$$

With:

D_{Feret}, Feret diameter is the distance between two parallel tangents of the particle at an arbitrary angle. The Feret diameters for a sufficient number of angles are calculated, and their maximum (or minimum) is selected. If a particle has an irregular shape, the Feret diameter varies more than with regularly shaped particles.

The sphericity (S) is defined in equation 6 and ranges from 0 to 1. The smaller the value, the more irregular the shape of the particle **is**. A sphericity value of 1 indicates a spherical particle.

$$S \ = \ P_{EQPC}(\mu\text{m})/P_{\text{real}}(\mu \ \text{m}) \tag{6}$$

With:
P_{EQPC}, the perimeter of a circle with the same area,
P_{real}, the real perimeter of particles.

Many trials and observations of particle shape parameters led to defining hibiscus fibers as all particles with elongation <0.5, aspect ratio <0.55, and sphericity <0.55. The elongation and aspect ratio allowed identifying some elongated particles and fibers. Sphericity parameters allowed refined searching and eliminated long or elliptical particles that are not fibers. The fiber per 100 g powder was defined in Equation 7:

$$\text{FR } (\%) = (\text{Fiber number/number of measured particle/} \\ \text{powder mass}) \ * \ 100 \tag{7}$$

2.3.5. Powder flowability

Powder flow behavior was analyzed using a FT4 powder rheometer (Freeman Technology, Worcestershire, United Kingdom) with 25 mm accessories.

2.3.5.1. Compressibility test. The compressibility test allows the measurement of the evolution of powder density as a function of the applied normal stress. To this end, three conditioning cycles were performed with a 23.5 mm blade followed by the split of the vessel (25 × 10 mL). The blade was replaced by a vented piston to subject the powder to nine levels of normal stress from 1 to 15 kPa. The normal stress was held constant for 60 **sec** to allow the powder to stabilize. The compressibility test allows obtaining compressed bulk density (CBD, expressed in $g.mL^{-1}$), which is the ratio between the powder mass and the volume of the powder bed after compression (Equation 8).

$$\text{CBD} \ = \ (\text{Split mass})/(\text{Volume after compression}) \tag{8}$$

2.3.5.2. Shear cell test. The shear cell test is a method allowing to determine the incipient shear stress required to put a consolidated powder that was previously at rest, in motion. Powder sample placed in a vessel (25 × 10 mL) underwent one conditioning cycle and was then compacted at 9 kPa. The vessel was split, and the shear cell test was then operated using a shear cell head. The powder was first re-compacted at 9 kPa to remove any disturbance caused by the split and to ensure that the sample surface was suitably consolidated. Then, a pre-shear was applied to achieve a steady state of the powder bed. After that, the shear cell head applied to the powder bed normal stresses from 7 to 3 kPa steps; and the incipient shear stress, which is the minimum shear stress necessary to cause the failure of the powder bed and make it flow, was recorded for each applied normal stress. The major principal stress (σ_1) and the unconfined yield stress (σ_c) were determined by the FT4 software using the yield-locus approach which permitted to calculate the flow factor (ff, Equation 9).

$$ff = \sigma_1/\sigma_c \tag{9}$$

Powder flowability can be deduced from flow factor values by using Jenike's classification (Jenike 1964):

- ff <1: not flowing
- 1 <ff <2: very cohesive
- 2 <ff <4: cohesive
- 4 <ff <10: easy flowing
- 10 <ff: free flowing

2.4. Powder reconstitution

2.4.1. Powder reconstitution parameters

Powder reconstitution was performed by stirring 0.5 g powder in 50 mL distilled water at 650 rpm for 30 min at 50 °C, which constitutes the optimum temperature for the extraction of anthocyanins from *Hibiscus sabdariffa* calyxes as proposed by Chumsri, Sirichote, and Itharat (2008).

2.4.2. Anthocyanins content determination

1.5 mL solution of reconstituted powder, which constitutes an aqueous extract, was taken and centrifuged (Minispin plus, Eppendorf AG, 22331 Hamburg, Germany) at 23 548 g for 30 **sec**. Red color intensity (CI, Equation 10) and browning index (BI, Equation 11) were followed by measuring the absorbance (A) of diluted extracts (dilution factor = 10) at wavelengths of 520 nm (A_{520}) and 430 nm (A_{430}) (Cisse 2010) using a UV/vis. spectrophotometer (Shimadzu UV 1280, Kyoto, Japan). Distilled water was used as the blank. The anthocyanins absorb at 520 nm and the browning molecules at 430 nm (Cisse 2010; Nguyen 2018).

$$CI = A_{520} \tag{10}$$

$$BI = A_{430}/A_{520} \tag{11}$$

2.4.3. Water-insoluble material

Water-insoluble material was quantified after powder reconstitution, according to a method adapted from Cisse (2010). The solution was centrifuged (Heraeus Megafuge 8 R, ThermoFisher Scientific, Germany) at 2268 g for 10 min to obtain 2 phases. The excess supernatant was removed and replaced with 35 mL distilled water at 50 °C. Solutions were vortexed at 2000 rpm for 10 s and then centrifuged using the same conditions as previously. The supernatant was removed, and the pellet was poured in a cup to be dried at 103 °C for 24 h. The dry matter was finally weighed, and the percentage of water-insoluble material was calculated according to Equation 12.

$$\text{Insoluble material } (\%) = m_{residual}/m_{powder} \times 100 \tag{12}$$

With:

- $m_{residual}$, the mass of water-insoluble material (g),
- m_{powder}, the mass of powder initially introduced for reconstitution (g).

2.4.4. Brix and pH

The pH of reconstituted powders was measured using a pH-meter (Mettler toledo, Five easy plus). Brix of solutions (90% of water and 10% powder) was measured at 20 °C using a hand-held refractometer (ATAGO, Master-M).

2.4.5. Statistical analysis

The statistical analyses were performed using XLSTAT 2019.4.2 (ADDINSOFT, France) add-on for Microsoft Excel software. The experiments were performed in triplicate. Reported values are means ± standard deviations. Data was subjected to a one-way analysis of variance (ANOVA) to determine if there were significant differences between means ($p < 0.05$). The Tukey's honestly significant difference (HSD) test was used to sort powders into groups significantly different between them. **A** non-parametric test, precisely the Kruskal–Wallis test was applied when the assumptions of ANOVA were not met. The Pearson correlation coefficient was also evaluated to measure the degree of linear correlation between two variables.

3. Results and discussion

3.1. Impact of processing parameters on powder properties

3.1.1. Water content

Sun-dried *Hibiscus sabdariffa* calyxes (18.50 g/100 g water content on wet basis), lost 18% of their initial water content after 1 h oven drying (T1) to reach 15.14 ± 0.40 g/100 g (Table 1). After 2 h oven drying (T2), the water content of calyxes was 14.24 ± 0.59 g/100 g, which corresponds to a 6% further reduction of initial water content compared to T1. Water activity was lowered by 16% ($a_w = 0.56 \pm 0.02$) compared to T0 after the first hour of oven drying (T1), then the calyxes reached 0.53 ± 0.01 water activity after 2 h oven drying (T2). Beyond 1 h drying time, the water content of the calyxes did not reduce **by** much because of the hardness of the calyx surfaces and the removal of the quasi totality of free water. The remaining water content was likely bound water. The T1 and T2 oven-dried calyxes were free of microbial growth ($a_w < 0.65$) but remained sensitive to enzymatic and non-enzymatic reactions and lipid oxidation (Labuza 1977). Sun-dried calyxes had the highest water activity of 0.65 ± 0.01 and were thus more prone to alteration by bacteria and fungi (Adebayo-Tayo and Samuel 2009), lipid oxidation, and enzymatic reactions.

The grinding step systematically led to a reduction of water content and water activity (Table 1). This reduction was greater for T0 calyxes: T0 powders ground at

Table 1. Physicochemical properties of *Hibiscus sabdariffa* calyx powders.

Samples	Sample acronym	Water content (g/100 g on wet basis)	Water activity (-)	Span (-)	Cohesion (kPa)	Flow factor (-)	Fiber (%) (on wet basis)
Calyxes	Dried calyxes (T0)	18.50 ± 0.36^{a}	0.65 ± 0.01^{a}	–	–	–	–
	Dried calyxes (T1)	15.14 ± 0.40^{b}	0.56 ± 0.02^{bc}	–	–	–	–
	Dried calyxes (T2)	14.24 ± 0.59^{de}	0.53 ± 0.01^{bc}	–	–	–	–
Powders	HS_T0_B10	15.49 ± 0.22^{bc}	0.55 ± 0.02^{bc}	2.11 ± 0.06^{e}	1.77 ± 0.05^{ab}	2.89 ± 0.1^{e}	1.45 ± 0.2^{a}
	HS_T1_B10	14.15 ± 0.03^{de}	0.59 ± 0.01^{c}	2.24 ± 0.06^{de}	1.62 ± 0.24^{abc}	3.28 ± 0.36^{cde}	0.56 ± 0.01^{b}
	HS_T2_B10	13.31 ± 0.02^{f}	0.45 ± 0.02^{d}	2.42 ± 0.1^{bcd}	1.2 ± 0.11^{bcd}	4.52 ± 0.32^{bc}	0.42 ± 0.03^{bcd}
	HS_T0_B12	15.80 ± 0.14^{b}	0.56 ± 0.01^{bc}	2.22 ± 0.05^{de}	1.11 ± 0.01^{cd}	4.39 ± 0.12^{bcd}	1.32 ± 0.14^{a}
	HS_T1_B12	14.19 ± 0.07^{de}	0.53 ± 0.00^{c}	2.56 ± 0.05^{abc}	1.11 ± 0.05^{cd}	4.56 ± 0.35^{bc}	0.53 ± 0.07^{bc}
	HS_T2_B12	13.29 ± 0.08^{f}	0.46 ± 0.02^{d}	2.56 ± 0.16^{abc}	0.82 ± 0.13^{d}	6.15 ± 0.97^{a}	0.26 ± 0.02^{d}
	HS_T0_B14	14.82 ± 0.12^{cd}	0.55 ± 0.02^{b}	2.31 ± 0.07^{cde}	1.98 ± 0.55^{a}	2.92 ± 0.66^{de}	1.21 ± 0.09^{a}
	HS_T1_B14	14.07 ± 0.05^{e}	0.53 ± 0.02^{c}	2.61 ± 0.06^{a}	1.00 ± 0.13^{cd}	4.97 ± 0.64^{ab}	0.28 ± 0.03^{cd}
	HS_T2_B14	13.14 ± 0.15^{f}	0.48 ± 0.01^{d}	2.80 ± 0.13^{a}	0.92 ± 0.10^{d}	5.5 ± 0.56^{ab}	0.27 ± 0.02^{cd}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p < 0.05$).

10,000 rpm (T0_B10) had a 16% lower water content than the unground T0 calyxes. Water content decreased by 2% when increasing the grinding frequency from 10,000 (B10) to 12,000 rpm (B12), and by 6% when the grinding frequency was raised from 12,000 (B12) to 14,000 rpm (B14).

To obtain powders, grinding T1 and T2 calyxes at B10 frequency resulted in a 7% decrease in water content. With higher grinding frequencies, the water content remained unchanged ($p > 0.05$). The powdered T0 calyxes also had a reduced water content when ground at B10. This water content was further reduced ($p < 0.05$) when increasing the grinding frequency from B10 to B14.

During grinding, the friction of the product between the rotor and the mill screen promotes local heating (Karam et al. 2016). Therefore, it was expected that friction would increase with grinding frequency. However, the grinding temperature only reached 30 °C in all experiments and did not vary with grinding frequency. Therefore, this temperature was not sufficient enough to be responsible for significant evaporation or a reduction in water content. On another note, grinding results in an increase in the specific surface area of the particles which would have favored the transfer of water to the ambient air, which could justify this reduction in water.

3.1.2. Particle size distribution

When increasing the drying duration, the particle sizes decrease whatever the grinding frequency (Figure 2). Significant size reductions were observed for all T1 powders compared to the reference powder (T0): 44, 50, and 52% for B10, B12, and B14, respectively. The particle size of all T2 powders did not significantly differ from T1 powders. Differences of 14, 7, and 5% between T1 and T2 powders were obtained for B10, B12, and B14, respectively. Therefore, 1 h oven drying had the most influence on grinding-induced particle size reduction. T0 (resp., T2) calyxes led to the largest (resp., smallest) median particle sizes in agreement with the Pearson coefficient, which shows a strong influence ($R = -0.83$) of drying duration on particle size. Upon drying-induced water removal, the material collapses and becomes tougher, more brittle, or more friable. All of this facilitates its grinding. Consequently, the powder with the smallest water content resulted in the finest powder as highlighted by Sui et al. (2019). This observation is confirmed by Deli et al. (2019), who reported that sun-dried *Hibiscus sabdariffa* calyxes ground at 12,000 rpm had a median particle size of 125.65 ± 2.08 µm and water content of 7.83 ± 0.5 g/100 g. Powders produced by Deli et al. (2019) – with the same equipment and in the same conditions as HS_T0_B12 powders of the present study – were finer, probably owing to their water content which was half of the hibiscus calyxes of the present study.

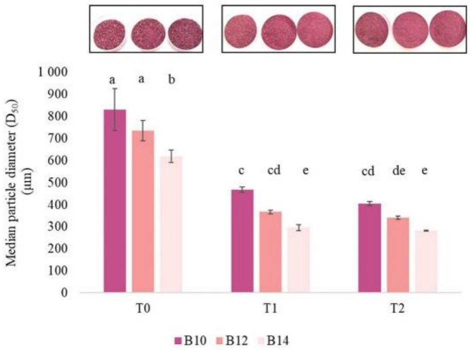

Figure 2. Median particle diameter of *Hibiscus sabdariffa* calyx powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

As expected, the median particles size decreased at a higher grinding frequency (Figure 2). For T0, T1, and T2 powders, the median particle size was reduced by 11, 22, and 16% respectively, from B10 to B12. From B12 to B14, the median particle decreased by 16, 20, and 17% for T0, T1, and T2 powders respectively. The *Hibiscus sabdariffa* calyx particles became finer at higher grinding frequency, which is consistent with okra seed powders studied by Waiss et al. (2020). Increasing the grinding frequency confers more kinetic energy to the material to be ground, favoring its break upon impact on the tooth-shaped rotor and **the** crushing between the rotor and the ring sieve. Moreover, using a higher grinding frequency at constant grinding duration increases the total number of rotations of the rotor, leading to finer products. The finest powder was obtained from calyxes that were oven-dried for 2 h and ground at 14,000 rpm (HS_T2_B14). As previously

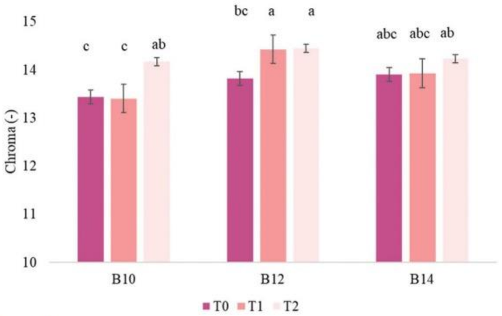

Figure 3. Impact of drying duration and grinding frequency on chroma of *Hibiscus sabdariffa* powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

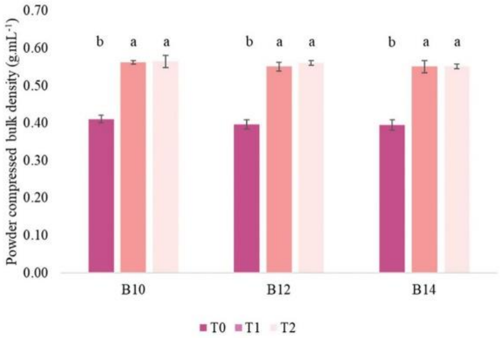

Figure 4. Impact of drying duration and grinding speed on compressed bulk density of *Hibiscus sabdariffa* calyx powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

mentioned, drying may increase calyx brittleness, thus favoring the grinding. All distributions were bimodal with span values ranging from 2.1 to 2.8 (Table 1), which points to the heterogeneity of *Hibiscus sabdariffa* calyx powders composed of two main populations: fine and large particles. Gnagne et al. (2017), Deli et al. (2019), and Waiss et al. (2020) showed that particle size distributions of powders issued from plant grinding **were** generally constituted of more than one population. For B12 and B14 powders, span values increased from T0 to T1 and did not significantly change with further drying duration. Grinding frequency also favored an increase in span for T1 and T2 powders, therefore the heterogeneity. The greater the grinding frequency, the finer the particles; and the greater the span, the more heterogeneous the powders.

3.1.3. Powder color

Drying duration affects powder color saturation, as reflected by chroma results (Figure 3). It was generally observed that chroma value increased with drying duration. An observation confirmed by the Pearson coefficient ($R = 0.58$) indicates a moderately positive influence of drying duration on chroma. Indeed, the longer the drying time, the more intense the powder color. Chroma did not change after 1 h oven drying for B10 powders but increased after 2 h. The chroma significantly ($p < 0.05$) increased from T0 to T1 for B12 powders, and became almost constant from T1 to T2. These results show that drying duration led to the discoloration of hibiscus calyx powders, caused by the formation of drying-induced oxidized products. Chroma value was also affected by the grinding frequency, as suspected, but only for T1 powders (Figure 3). Chroma of these powders increased by 8% from B10 to B12 and did not significantly change from B12 to B14. Higher grinding frequency favored more intense powders color, which is a result of the grinding-induced increase in particle specific area.

3.1.4. Powder flowability

Compressed bulk densities of T0 powders were significantly lower than those of T1 and T2 powders, depending on the grinding frequency (Figure 4). This result was expected since T0 powders had higher interparticular porosity (related to their irregular and large particles) than T1 and T2 powders (Figure 5). T1 and T2 powders had similar bulk densities ranging from 0.55 ± 0.011 to $0.56 \pm 0.014\,\text{g.mL}^{-1}$, which could be attributed to the similarity of their particle size distributions. Moreover, grinding frequency seemed to not have a significant influence on powder bulk density. The compressibility results are depicted in Figure 6(a). T1 and T2 powders had similar relatively low compressibility, whereas T0 powders were more compressible. The compressibility of T1 and T2 powders at 15 kPa normal stress was 12% for B10 and B12 powders and 15% for B14 powders. This low compressibility of T1 and T2 powders may be an indication of their better flowability than T0 powders (Gnagne et al. 2017). The close compressibilities of T1 and T2 powders were likely to result from their similar particle size distributions. Compressibility of T0 powders was more important: 21, 24, and 23% for B10, B12, and B14 batches, respectively. The higher water content of T0 powders may contribute to their higher compressibility. Therefore, water has a plasticizing effect on solid materials, which increases their deformability upon compression and increases the stickiness of particles and their cohesion. This in turn enhances powder compressibility (Fitzpatrick et al. 2007; Zafar et al. 2017). Powder compressibility was also affected by grinding frequency. For T1 and T2 powders, compressibility was similar for B10 and B12 batches and increased for the B14 batch. This may be explained by the finest particles (i.e., lower median particle size) and the higher heterogeneity (i.e., higher span) of T1_B14 and T2_B14 powders. Indeed, more heterogeneous powders generally have higher compressibility (Petit et al. 2017). Since compressibility is representative of the intergranular forces and therefore, indirectly of the cohesion of powders (Petit et al. 2017), it is important to carefully handle compressible powders to avoid caking during handling, packaging, or storage.

The evolution of incipient shear stress when changing the applied normal stress is presented in Figure 6(b). The application of normal stress to the powder bed causes particle

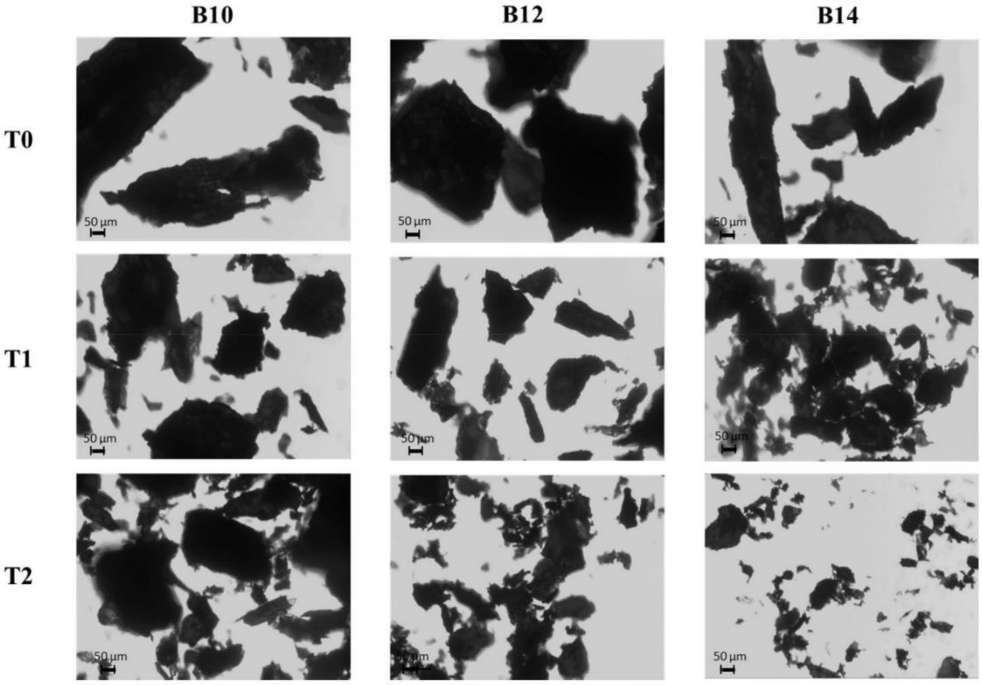

Figure 5. Morphological observation of particle surface of *Hibiscus sabdariffa* powders.

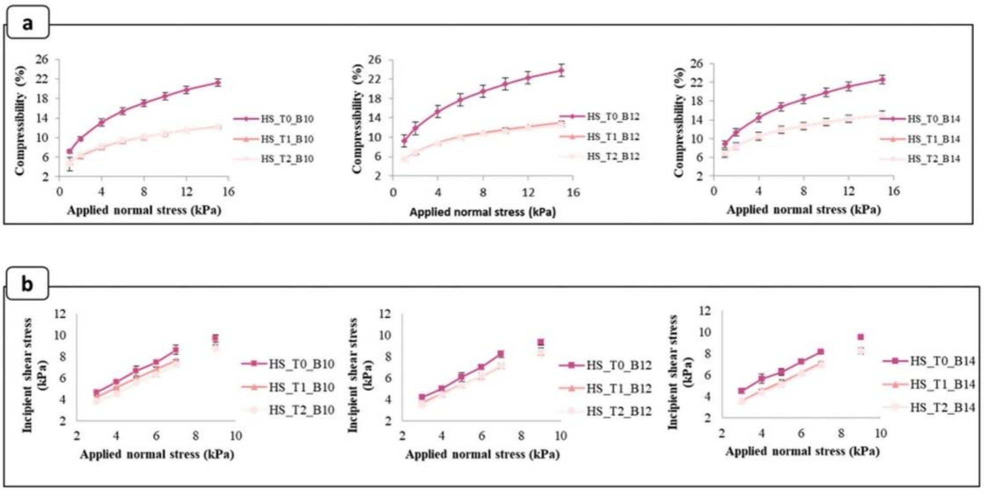

Figure 6. Compressibility (a) and yield locus (b) of *Hibiscus sabdariffa* calyx powders at various applied normal stresses.

deformations leading to a decrease in the interparticle distance and an increase in the number of interparticle contact points, which enhances powder cohesion and increases incipient shear stress (Hartmann and Palzer 2011). Incipient shear stresses of T1 and T2 at a given applied normal stress were similar. T0 powders had higher incipient stresses than T1 and T2 powders for all grinding frequencies. This difference is more important for B14 powders for which T1 and T2 powders were significantly less cohesive than T0 powders (Table 1). This indicates that T0 powders had poorer flowability than T1 and T2 powders. The poor flowability of T0 powders may be due to their greater abundance of fiber and/or their greater water content. The latter often enhances powder cohesion and is made possible because of its plasticizing effect. Moreover, Petit et al. (2017) reported higher interparticle contact points in powders rich in rod-shaped particles, such as fiber particles, thus favoring more particle cohesion. T1 and T2 powders, therefore, have better flow properties than T0 powders, which were cohesive and more sensitive to caking. Pearson correlation ($R = -0.60$) showed a reduction of cohesion and a better flowability of heterogeneous T1 and T2 powders. The fine particles on the surface of the large particles could limit the interparticle contact and friction and improve the flowability; this is the ball rolling effect (Petit et al. 2017). According to Jenike's classification (Table 1), HS_T0_B10, HS_T1_B10, and HS_T0_B14 were cohesive, whereas all other powders felt into the easy-flowing category.

3.2. Impact of processing parameters on reconstituted powders

It can first be observed that an increase in the drying duration enhances the red color intensity of solutions of reconstituted B10 powders (Figure 7). For the B10 batch, the color intensity from T2 powder was increased by 8% compared to T0 powder. This may be explained by the higher acidity of these extracts (Table 2), offering better stability of the anthocyanins, therefore, a higher red color intensity (Sinela 2016) and/or the higher proportion of products of Maillard reaction in powders having undergone a longer drying, as highlighted by chroma values (Figure 3). For B12 and B14 batches, the red color intensity of solutions of reconstituted powders increased in the following order: T1 < T2 < T0. The red color intensity was related to the concentration of anthocyanins. Therefore, the anthocyanin concentration of B12 and B14 powders may be decreased during the drying steps. Many studies reported a reduction of *Hibiscus sabdariffa* anthocyanins content during drying or extraction (Chumsri, Sirichote, and Itharat 2008; Daniel et al. 2012; Loyola Arenas et al. 2016; Sinela 2016; Djaeni et al. 2018). They highlighted a decline of the anthocyanin content with increments of temperature and/or time, thus attracting attention to the thermal sensitivity of anthocyanin molecules during manufacturing. The alteration of anthocyanins should also depend on the characteristics of the matrix in addition to the process parameters. The red color intensity significantly increased with the grinding frequency (Figure 7) for T0 powders only; an increase of 12% was observed from T0_B10 to T0_B12 powders, and this increase was less significant ($p > 0.05$) for T0_B14 powder, characterized by an increase of only 2% compared to T0_B12 powder. This trend was confirmed by the Pearson coefficient, indicating a positive moderate influence ($R = 0.51$) of grinding frequency on color intensity. The specific surface area of particles increased during the grinding process (Singh, Ghodki, and Goswami 2018), making powder constituents such as anthocyanins, which are responsible for the red color and more accessible to water, thus improving extraction efficiency. The red color intensity of T1 and T2 powders remained quasi constant when increasing the grinding frequency. It could be due to the drying process, during which the anthocyanin compounds were slightly altered. Moreover, the content of these compounds may have declined over time because of their exposure temperature, oxygen, or light (Jackman et al. 1987; Singh, Ghodki, and Goswami 2018; Tham et al. 2018; Deli et al. 2019), which leads to the production of browning compounds. The presence of the latter in T1 and T2 powders (Table 2) may be resulting from anthocyanin alteration. As indicated by the Pearson coefficient correlation ($R = 0.594$), browning compounds were partly responsible for the increase in chroma, therefore for the modification of powder color.

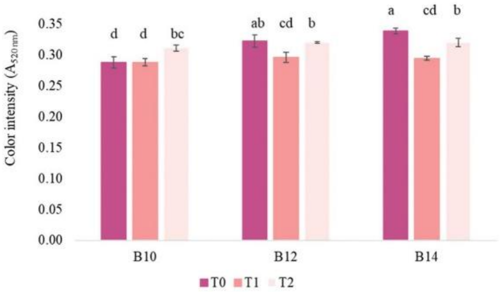

Figure 7. Impact of drying duration and grinding frequency on color intensity (absorbance at 520 nm) of solutions of reconstituted *Hibiscus sabdariffa* powders (bars topped by different letters are significantly different according to Tukey's HSD test ($n = 3$, $p < 0.05$)).

Table 2. Chemical properties of solutions of reconstituted powders of *Hibiscus sabdariffa* calyxes.

Sample acronym	pH (-)	Brix (°Brix)	Insoluble Material (%) (on wet basis)	Browning index (-)
HS_T0_B10	2.64 ± 0.03^{ab}	5.33 ± 0.58^{a}	40.25 ± 3.27^{ab}	0.45 ± 0.04^{a}
HS_T1_B10	2.64 ± 0.01^{ab}	5.00 ± 0.00^{a}	38.32 ± 0.69^{ab}	0.44 ± 0.01^{a}
HS_T2_B10	2.59 ± 0.01^{b}	5.33 ± 0.58^{a}	42.01 ± 1.57^{a}	0.44 ± 0.02^{a}
HS_T0_B12	2.65 ± 0.01^{a}	6.00 ± 0.00^{a}	40.27 ± 2.49^{ab}	0.47 ± 0.01^{a}
HS_T1_B12	2.63 ± 0.00^{ab}	5.33 ± 0.58^{a}	38.00 ± 0.35^{ab}	0.46 ± 0.01^{a}
HS_T2_B12	2.61 ± 0.02^{ab}	6.00 ± 0.00^{a}	38.01 ± 0.96^{ab}	0.45 ± 0.01^{a}
HS_T0_B14	2.62 ± 0.00^{ab}	6.00 ± 0.00^{a}	39.55 ± 0.85^{ab}	0.44 ± 0.01^{a}
HS_T1_B14	2.63 ± 0.01^{ab}	6.00 ± 0.00^{a}	38.05 ± 0.94^{ab}	0.45 ± 0.01^{a}
HS_T2_B14	2.62 ± 0.02^{ab}	6.00 ± 0.00^{a}	36.19 ± 1.03^{b}	0.44 ± 0.02^{a}

Means ± standard deviations with different letters within the same column significantly differed according to Tukey's HSD test ($n = 3$, $p < 0.05$).

The insoluble material (IM) is presented in Table 2. A non-significant decrease in IM of calyx powders resulted in high drying duration. On the contrary, increasing in grinding frequency from B10 to B14 resulted in a significant reduction of T2 powders IM. Hibiscus calyxes are rich in fiber (Cisse 2010; Deli et al. 2019), which makes the grinding difficult (Becker et al. 2016; Zaiter et al. 2016), hence the need for powerful grinding. Important influence of processing parameters on powder IM was obtained due to the particle size reduction favored at higher drying duration and grinding frequency. Brix of reconstituted powders increased significantly only with the grinding frequency from B10 to B14 for T1 powders (Table 2), confirming that higher grinding frequency improves the solubility of reconstituted powder. Powder solubility obtained from finer powders enhanced the availability of components but remained incomplete due to the presence of fiber. This may cause turbidity or precipitation of solutions at rest, which may be an undesirable effect in industry or not be appreciated by consumers.

4. Conclusion

Water content, fiber rate, particle size and shape, heterogeneity, and cohesion are relevant to understand the flowability of hibiscus powder, induced by drying coupled with grinding. Complementary drying times to sun-drying allow the production of almost identical powder with good rheological behavior. Despite this good flowability, it is important to note that the anthocyanins may be altered at high drying duration, particularly in fine powders where specific surface area is more important. Therefore, the combined effect of drying and grinding on powders leads to the right adjustment of drying duration and grinding when powdering *Hibiscus sabdariffa* calyxes because the preservation of its quality – more precisely, its anthocyanin content and powder flowability are relevant end-use functionalities for consumers. On this basis, powders T1_B12, T1_B14, T2_B12, T2_B14 are of particular interest because they combine solubility, ease flowing, have a relatively good red color, and are low water content for a long shelf life. In future work, and in addition to the red color, the anthocyanin content and the antioxidant activity have to be assessed to ensure the nutritional quality of the powders.

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DOSSIER

Valorisation des calices d'Hibiscus sabdariffa riches en anthocyanes pour la formulation de boissons

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L'Hibiscus sabdariffa (hibiscus) contient des polyphénols et des molécules d'anthocyane qui sont à la fois des composés antioxydants et responsables de la couleur rouge attrayante de ses calices. Cependant, l'hibiscus est une plante tropicale et saisonnière dont les calices sont très périssables, et les composés anthocyaniques thermosensibles et instables en milieu aqueux. Dans ce contexte, la transformation des calices en poudre permet de stabiliser la fleur et faciliter l'accessibilité à ses bienfaits pour la santé, à condition que le procédé soit maîtrisé. L'un des meilleurs moyens de préserver les propriétés d'origine est d'obtenir des produits peu transformés « minimally processed products » en combinant le séchage à l'étuve, le broyage et le fractionnement par tamisage. Ce procédé a été étudié dans ce travail et les propriétés physicochimiques, et fonctionnelles des poudres (extractibilité des biomolécules) ont été systématiquement évaluées dans un but de formulation de boisson. L'enjeu d'un tel procédé est la facilité de sa mise en place applicable à tous végétaux, la facilité d'usage et surtout la préservation de la qualité des poudres par amélioration de la disponibilité et l'accessibilité des biomolécules. A l'issue de ce travail, ont été obtenues en un procédé unique, deux poudres : une poudre fine et une poudre grossière dont les propriétés physicochimiques et d'extraction singulières ont permis d'envisager des modes d'utilisations différenciés et donc des méthodes de valorisation adaptées à chaque poudre.

Mots clés : Poudre d'Hibiscus sabdariffa, « minimally processed product », anthocyane, activité antioxydante.

ABSTRACT

Hibiscus sabdariffa (hibiscus) is rich in polyphenol and anthocyanin molecules that are both antioxidant compounds and responsible for the attractive red color of its calyxes. However, this plant is tropical and annual, with perishable calyxes and heat-sensitive anthocyanins that are not stable in water. In this context, calyx transformation into powder allow stabilizing the flower and facilitate the accessibility to its health benefits provided that the process is controlled. One of the best way to preserve original properties of food powder is to obtain «minimally processed foods» by combining oven drying, grinding and sieving. This process has been studied in this work and the powder physicochemical and functional properties (biomolecule extractability) have been systematically evaluated for the purpose of beverage formulation. The challenge of such a process is the ease of its implementation applicable to all plants, and the powder quality preservation by improving biomolecule availability and accessibility. This work resulted in production of two powders in a only one process: fine powder (< 212 µm) and coarse powder (> 212 µm) whose singular physicochemical and extraction properties allow to propose different use modes and valorization methods adapted to each powder.

INTRODUCTION

De nos jours, l'alimentation ne se résume plus uniquement en un simple apport énergétique mais l'intérêt des consommateurs est également porté sur les bénéfices santé que les aliments peuvent apporter. Par exemple, les consommateurs se dirigent de plus en plus vers des aliments naturels fonctionnels prêts à l'emploi et riches en antioxydants [1]

L'intérêt porté aux calices d'hibiscus rouge repose principalement sur leur composition chimique, en particulier leur richesse en anthocyane et phénols. Le goût fruité acide, la coloration rouge en plus de l'activité antioxydante des calices constituent les principaux potentiels marketing, les critères d'achat et de consommation de l'hibiscus.

Pour utiliser les calices d'hibiscus, différentes méthodes de stabilisation et de transformation en poudre ont été étudiées (avec pour objectif la réduction de la teneur en eau) : le séchage des calices entiers, l'extraction suivie de la production de poudre par séchage par atomisation ou par broyage. Pour leur employabilité, les poudres d'hibiscus sont reconstituées avec de l'eau avant usage, dans le but de préparer les boissons et infusions ou pour rehausser la couleur des préparations. Cette étape de reconstitution permettant d'extraire les molécules solubles est indispensable à leur utilisation. Par conséquent l'optimisation des productions de boissons passe inéluctablement par la maîtrise de l'extraction des biomolécules.

Figure 1 : Hibiscus sabdariffa cultivé à Korhogo en Côte d'Ivoire (décembre 2020), a – plante, b – fleurs, c – calices.

Impact of processing parameters on the functional and physicochemical properties of Hibiscus *sabdariffa* calyx powders

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Introduction

Hibiscus *sabdariffa* is an herbaceous plant cultivated in many tropical and subtropical countries. One edible part is the calyxes, a rich source of anthocyanins responsible of the red colour. These calyxes are widely used as infusion or fresh juice from dried calyxes or powder.

Drying or powdering reduce the humidity (90 % HR) allowing a good preservation and facilitate their use. However the powder properties as well as the beverage are modified according to the process that may cause anthocyanins degradation.

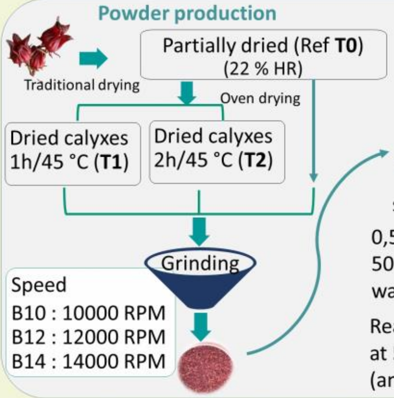
Objectives

The objectives are :

- The implementation of powder production processes and chemical, physical, functional properties characterization of Hibiscus *sabdariffa* powders
- The study of the influence of processing parameters on the physicochemical properties of powders
- The impact of the powder properties on beverage formulation

Materials & Methods

Powder production



Chemical

***Color test**

Spectrophotometer

0,5 g of powder in 50 ml distilled water

Read absorbance at **520 nm** (anthocyanins)

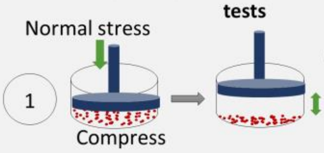
Physical

***Particles size and shape**

QICPIC

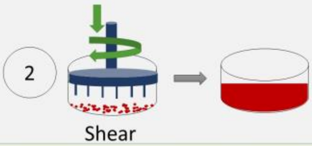
Functional

***Flowability : compressibility (1) and shear stress (2) tests**



Compressibility = % volume reduction

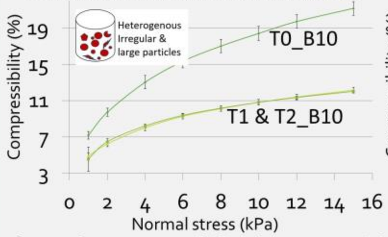
Normal stress & rotation



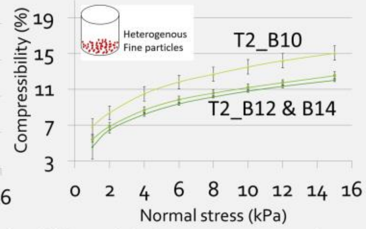
The **shear stress** that results in the flow of the powder bed is measured

Results

***Impact of drying duration on compressibility**

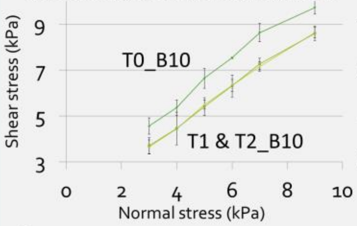


*** Impact of grinding speed on compressibility**



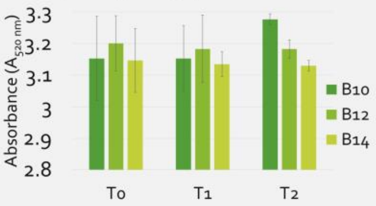
→Powder compressibility decreases with additional drying treatment and with increasing grinding speed.

***Impact of drying duration on shear stress**



→Powders obtained from partially dried calyxes (T0) are cohesive. They are difficult to flow contrary to others.

***Impact of grinding speed on absorbance**



→Coloration, an anthocyanins indicator, is better preserved at low grinding speed.

Conclusion

- The powder physicochemical properties are strongly dependent on the processing factors : drying duration and grinding speed.
- Preserving anthocyanins is of paramount importance and it is possible with a low grinding speed.
- However at this low parameter, the particles powders are large , irregular and heterogenous.
- Future work :
  Evaluate how the formulation of the beverage is impacted by powder physicochemical properties

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Valorization of whole calyxes of *Hibiscus sabdariffa* by production of powders with high nutritional value

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INTRODUCTION

Consumers are increasingly interested in functional natural foods such as vegetable beverages ready to use and rich in antioxidants compounds, including *Hibiscus sabdariffa* (hibiscus) calyx drink. Hibiscus contains polyphenols and anthocyanin molecules that are both an antioxidant and responsible for its appealing red color [1]. However, the extraction of plant juices produces a lot of waste [2], composed of insoluble materials still rich in bioactive compounds to be valorized. In addition, hibiscus is a seasonal plant that grows only in tropical regions, and anthocyanin compound is not stable in aqueous medium. In this context, processing calyxes into powder easy to use is an alternative to stabilize, facilitate accessibility to its health benefits, and reduce the losses related to extraction.

OBJECTIVES

1 Reduce calyxes into powder

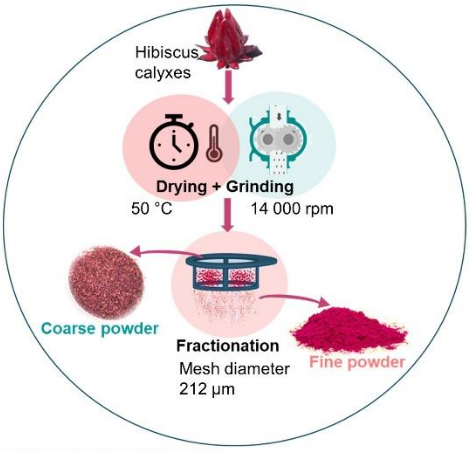
2 Maximize the extraction of active biomolecules

3 Ensure a good powder flowability

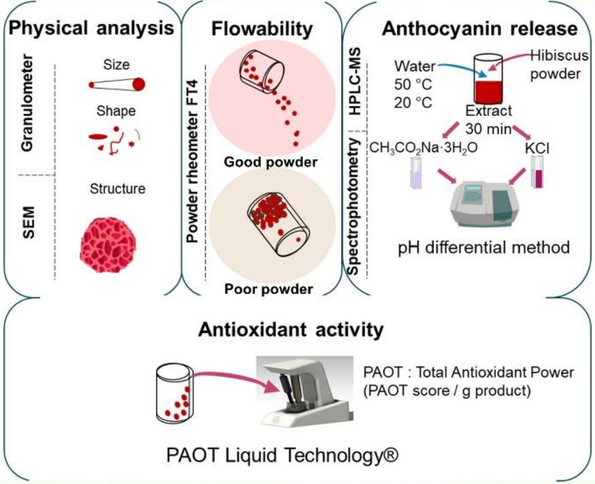
4 Reduce insoluble material

MATERIAL & METHODS

Powder production

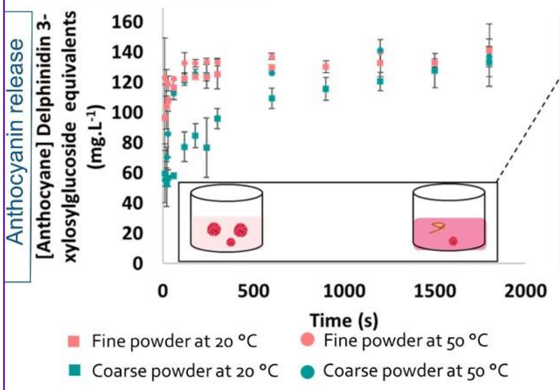


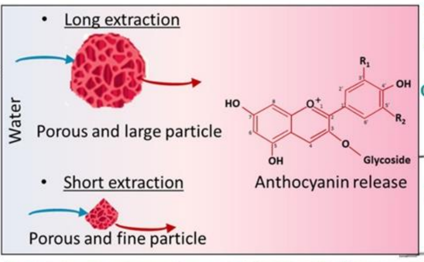
Analysis

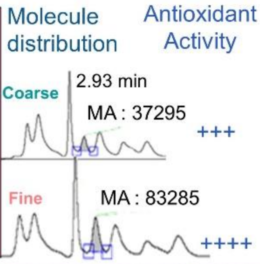


RESULTS & DISCUSSION

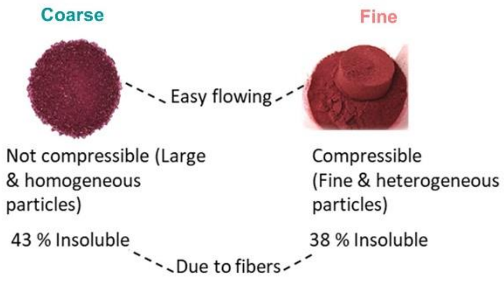
Anthocyanin release







Flowability



CONCLUSION & PERSPECTIVES

- The whole vegetable part is valorized by producing two powders with single flowability and high nutritional value in a one process
- Fine powder is an add-value product because of its highest antioxidant power
- Some optimal modes of uses are specified:
  At **50 °C**, (i) fine powder is instantaneous and (ii) coarse powder requires infusions time (2 min); (iii) **at 20 °C,** maximal extraction is also possible before 10 min, and allow avoiding risks of heat sensitive compounds degradation
- Insoluble material of fine powders makes drinks cloudy that may be accepted by consumers, while that of coarse powders is still considered as waste

🔍 Insolubility can be further improved by using density method to remove fiber particles that can then be reused for composting

🔍 Encapsulation step could also be required to optimize the anthocyanin stabilization

[1] Monteiro, M.J.P., Costa, A.I.A., Tomlins, K.I., Pintado, M.E., 2019. Quality Improvement and New Product Development in the Hibiscus Beverage Industry, in: Processing and Sustainability of Beverages. Elsevier, pp. 139–183. https://doi.org/10.1016/B978-0-12-815259-1.00005-7

[2] Mármol, I., Quero, J., Ibarz, R., Ferreira-Santos, P., Teixeira, J.A., Rocha, C.M.R., Pérez-Fernández, M., García-Juiz, S., Osada, J., Martín-Belloso, O., Rodríguez-Yoldi, M.J., 2021. Valorization of agro-food by-products and their potential therapeutic applications. Food and Bioproducts Processing 128, 247–258. https://doi.org/10.1016/j.fbp.2021.06.003

Résumé / Abstract

Résumé

L'*Hibiscus sabdariffa* (hibiscus) est une plante dont les calices comestibles, contiennent des polyphénols et des molécules d'anthocyane qui sont à la fois des composés antioxydants et responsables de leur couleur rouge attrayante pour le consommateur. Ces particularités constituent un fort potentiel santé qui répond aux demandes actuelles du marché et un atout économique pour les industries. Cependant, cette plante tropicale et saisonnière dont les calices riches en eau, sont très périssables, sensibles à l’humidité et à la chaleur, et les molécules d’intérêt (anthocyanes) sont thermosensibles et instables en milieu aqueux. Dans ce contexte, la transformation des calices en poudre permet, en plus d’optimiser le coût du transport et l’espace de stockage, de stabiliser les calices en assurant une longue durée de conservation du produit, de faciliter l'accessibilité aux biomolécules et donc à ses bienfaits pour la santé, ceci, à condition que le procédé soit maîtrisé. L'un des meilleurs moyens de préserver les propriétés nutritionnelles d'origine (teneur en anthocyanes, activité antioxydante) des calices est d’obtenir des produits peu transformés ou « minimally processed products » en combinant le séchage à l’étuve (contrôlé), le broyage et le fractionnement par tamisage. Ce procédé a été étudié dans ce travail et les propriétés physicochimiques (granulométrie, forme, structure, porosité, proportion de fibre, teneur en anthocyanes, activité antioxydante) et fonctionnelles des poudres (écoulement, reconstitution, extractibilité des biomolécules) ont été systématiquement évaluées dans un but de formulation de boisson. L’intérêt d’un tel procédé est la facilité de sa mise en œuvre, applicable à tous végétaux, la facilité d'usage de la poudre, l’amélioration de la disponibilité et l’accessibilité des biomolécules. Ce travail a permis d’identifier l’impact du séchage solaire et du broyage sur les propriétés physicochimiques des poudres, ces dernières impactant directement les propriétés fonctionnelles. La substitution du séchage solaire par le séchage contrôlé à l’étuve et un fractionnement par tamisage supplémentaire après le broyage ont permis d’améliorer les propriétés fonctionnelles des poudres. Ces propriétés singulières des poudres ainsi obtenues, ont permis de distinguer des applications qui leur sont propres.

Mots clés : Poudre d’*Hibiscus sabdariffa*, « minimally processed product », anthocyane, activité antioxydante, écoulement, reconstitution, extractibilité des biomolécules.

Abstract

Hibiscus sabdariffa (hibiscus) is a plant with edible calyxes containing polyphenol and anthocyanin molecules that are both antioxidant compounds and responsible for their attractive red color for consumers. These particularities constitute a good health potential that meets the current market demands, and an economic potential for industries. However, this tropical and seasonal plant whose water-rich calyxes are highly perishable, sensitive to humidity and heat, and whose the interesting molecules (anthocyanins) are heat-sensitive and unstable in aqueous medium. In this context, the calyx transformation into powder allows, besides optimizing the transport cost and storage space, to stabilize the calyxes by ensuring a long product shelf-life, and to improve the biomolecule accessibility. This allows to benefit to its health assets provided that the process is controlled. One of the best ways to preserve the calyx original nutritional properties (anthocyanin content, antioxidant activity) is to obtain minimally processed products by combining controlled oven-drying, grinding and fractionation by sieving. This process was studied in this work and the powder physicochemical properties (particle size and shape, structure, porosity, fiber proportion, anthocyanin content, antioxidant activity) and functional properties (flowability, reconstitution, biomolecule extractability) were systematically evaluated for drink formulation. The interest of such a process is the ease of its implementation applicable to all plants, the powder ease of use, the improvement of the biomolecule availability and accessibility. This work allows identifying the impact of sun-drying and grinding on the powder physicochemical properties, the latter impacting the functional properties. The substitution of sun-drying by controlled oven-drying and an additional fractionation by sieving preceded by grinding allowed improving the powder functional properties. These powder functional properties were singular and allowed to distinguish specific applications for each powder type (fine or coarse powders).

Keywords: *Hibiscus sabdariffa* powder, minimally processed product, anthocyanin, antioxidant activity, flowability, reconstitution, biomolecule extractability.