氏名（本籍）	Meryem BOUHOUTE （モロッコ）
学位の種類	博士（食料革新学）
学位記番号	博甲第 9719 号
学位授与年月	令和 2 年 9 月 25 日
学位授与の要件	学位規則 第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Utilization of Surface-active Compounds and Microfibrillated Cellulose Particles from Argan Processing Residue as Sustainable Natural Emulsifiers (持続可能な天然乳化剤としてのアルガン加工残渣からの界面活性成分およびミクロフィブリル化セルロース粒子の利用)

	（職名）	（学位）	（氏名）
主査	筑波大学教授（協働大学院）	博士（農学）	小林 功
副査	筑波大学准教授	博士（農学）	Marcos Antonio das NEVES
副査	筑波大学教授	博士（農学）	礒田 博子
副査	筑波大学特命教授	工学博士	中嶋 光敏

Abstract of thesis

The utilization of natural ingredients derived from biomass is a promising path toward sustainability. In recent years, consumers are more aware about the resources and ingredients formulating their products and the challenges facing today's food engineering field are to provide healthy sustainable products while regaining consumer trust. Sustainability approach is based on three main pillars, environment, society and economy. For a product to be sustainable, it has to satisfy those three pillars. In addition, the development of functional food, dietary supplements, nutraceuticals and product reformulation for favorable health impact (salt reduction, calorie reduction) for an aging population is also a challenge toward healthiness. Finally, to regain consumer trust, food formulations require the incorporation of natural ingredients and providing clean label products.

Considering the above-mentioned aspects, the choice of argan oil industry made a lot of sense for this study to tackle the challenges of product formulation. This industry is unique to the Moroccan country because the species *Argania spinosa* (L.) Skeels is endemic to its desert. Argan tree plays an important environmental role in the region, because of its high resistance to environmental stress (water draught, arid land) therefore procuring a barrier to desertification and erosion of the soil. Argan plays also an important socio-economical role in the region, considering that argan is a wild species that have been present for centuries in southwestern Morocco. Today, 2-3 million people rely on argan tree as a source of income, in fact, all the parts of the tree are used today by the local population. The main product of argan fruit is the oil; however, this product represents only 3% of the total mass of the fruit. The remaining are argan press cake,

argan pulp and argan shell, which are considered by-products and are used by the locals as cattle feed (press cake and pulp) or burned for heat generation (shell).

Argan shell is a lignocellulosic biomass rich in fibers, which led to few researches centered toward this material to prepare composites or to be used as biochar. However, considering that argan oil made a real breakthrough to international markets (argan oil is known for its unique cosmetic and edible features), this thesis was inspired to valorize argan by-product (argan shell) within food, cosmetic or pharmaceutic industries.

Emulsifiers global market represents $8.4 billion and is divided between food market (30%), cosmetics (20%) and pharmaceutic and agrochemicals (50%). This market is expected to grow by 5.6 points in 2050. Mostly, when considering emulsifiers, it is more common to find a synthetic emulsifier used rather than a natural one. In fact, synthetic emulsifiers represent 70% of emulsifiers against only 30% of natural emulsifiers in the global market. The reason behind this difference is the inhomogeneous and high cost of natural emulsifiers when compared to their synthetic counterparts. Therefore, industrial are less attracted to their utilization when formulating products. On the contrary, this trend is now changing, and industrials and manufacturers have to adapt to the new aspirations of consumers, thus, providing acceptable products for them to use.

Oil-in-water (O/W) emulsion is a system encountered in many commercial products such as food, cosmetic and pharmaceutic industries. This system is thermodynamically unstable consisting of two immiscible liquids, one dispersed into the other, that would rapidly separate to the initial homogenized components in the absence of stabilizers. Emulsion stabilizers can be categorized into surface-active emulsifiers (surfactants) and thickening or gelling agents. Furthermore, solid particles were also described to stabilize emulsions forming the so-called “Pickering emulsions” that were first reported by Pickering (1907). The author focused on identify a new natural source of surface-active compounds and microfibrillated cellulose, and utilize them as emulsifier.

The author highlighted an exhaustive extraction of natural compounds from argan shell biomass and applied them in the formulation of O/W emulsions. The first extraction consisted on solid-liquid extraction using aqueous-ethanol as solvent. Five extracts were characterized in this study then compared for their emulsifying performance. The obtained residue of aqueous-ethanol extraction was used for the preparation and purification of cellulose. The pulping process consisted of alkali treatment and the effect of four different concentrations of sodium hydroxide on argan shell purified cellulose was studied. After reaching satisfying properties of argan shell cellulose, microfibrillated cellulose was prepared using mechanical disintegration.

First, the author conducted a characterization of the extracted compounds using spectrophotometric methods, Fourier Transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), interfacial tension and contact angle, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Then, the author focused on the utilization of the identified compounds to formulate and stabilize O/W emulsions, this involved high energy emulsification and monitoring physical stability under different environmental stress conditions. The results were successful and led to the formulation of emulsions using natural emulsifiers from argan shell.

Emulsions formulation and stability using natural emulsifiers are varying considerably due to the source and the type of surface-active compounds. This is even more the case when using crude extracts from plant material as emulsion stabilizers. Mostly, interfacial tension and composition are used to confirm the tendency of an emulsifier to formulate a stable emulsion. However, systemic studies also offer more reflection on the mechanism behind a successful stability. The author revealed the possibility of using crude argan shell extracts as natural emulsifiers. It was confirmed that with the same starting material, under different extraction conditions, all extracts could stabilize submicron emulsions with a highly negative charge and good physical

stability despite the variation in the composition of surface-active compounds. However only one argan shell extract (20-ASE, extracted using 20% ethanol) could form an emulsion with the lowest mean droplet size $d_{4,3}$ < 200 nm. This means that the whole composition contributes to emulsion formation when using complex mixtures of surface-active compounds and emulsion trials are needed in order to reveal this tendency.

The use of argan shell microfibrillated cellulose (AS-MFC) as O/W emulsions stabilizer was investigated. The effect of particles concentration was assessed and led to long term stability (15 days) of O/W emulsions at high concentration of AS-MFC confirmed by droplet size ($d_{4,3}$) and creaming index. The author reported the oil concentration suitable to reach the maximum volume of emulsion using 1% w/w AS-MFC. The results show AS-MFC could stabilize 70% w/w medium-chain triacylglycerol (MCT) oil. Finally, confocal laser scanning microscopy (CLSM) shows the adsorption of AS-MFC at the oil-water interface and the formation of a 3D network surrounding oil droplets by larger fibrils.

Based on the findings obtained from this research, the author provides evidence to predict an added value of argan shell for food, cosmetic or pharmaceutic industries. However, more research is required regarding toxicity of the compounds extracted from argan shell. In addition, for an industrial use, an economical based study is required for scaling up the production and reaching homogeneous compounds ready for utilization by the above-mentioned industries.

Besides the industrial application of surface-active compounds and microfibrillated cellulose, there are many scientific topics in which argan shell may offer new research opportunities. First, the chemical stability of bio-active compounds encapsulated by surface-active compounds or microfibrillated cellulose could be elucidated. Next, digestive stability of emulsions can be studied. Furthermore, it would be interesting to study the interfacial composition of emulsions stabilized by argan shell extracts. Another interesting topic would be the complexation of microfibrillated cellulose and surface-active compounds to deliver enhanced emulsifying properties.

Abstract of assessment result

【Review】

Argan (*Argania spinosa*), an endemic plant species from Morocco, produces fruits consisting of nearly 3 wt% oil and around 50 wt% shells, among other components. Considering the large amount of shells generated during argan oil processing, and the fact that those shells are mostly used as a source of energy by combustion, despite its potential in the production of secondary metabolites, this study focused on evaluating the emulsifying ability of products obtained from argan shells. In the first part of the study, the applicant evaluated the emulsifying ability of aqueous-ethanolic crude extracts from argan shell in oil-in-water (O/W) emulsions. This research focused particularly on the effect of argan shell composition, and interfacial properties and their impact on the formulated O/W emulsions droplet size. The results indicated that emulsions stabilized solely using argan shell extract had mean droplet size ($d_{4,3}$) between 200 nm and 440 nm, according to the concentration of ethanol using in the extract process, and those emulsions were stable up to 30 days even when stored at room temperature. In the second part of the study, the applicant prepared microfibrillated cellulose (MFC) from argan shells, by chemically purifying cellulose using alkali and bleaching treatments, then mechanical disintegration via high-pressure homogenization to isolate fibrils of cellulose. The emulsifying ability of MFC as emulsifier in oil-in-water emulsions was also investigated. The experimental results indicated the considerable emulsifying ability of MFC, as demonstrated by the long-term stability of the emulsions prepared using MFC solely as emulsifier. It was suggested that the stabilization mechanism involved in emulsion stabilization by argan shell crude extracts may be attributed to the synergic effect of surface-active compounds found in the extracts. It was also suggested that emulsion stabilization by MFC may be based on Pickering emulsion.

【Result】

The final examination committee conducted a meeting as a final examination on June 16, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during the question-and-answer session. All the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Food Innovation.