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CICLO XXXIII

Improvement of the use of food resources of African countries

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Dedicated to my two beloved Mums,
Nonna Pina and Luigina.

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Abstract

The world is not on track to reach the 2025 Global Nutrition Targets, nor the 2030 Sustainable Development Goals (SDGs). The number of hungry people in the world has been rising steadily since 2014, with the highest prevalence of undernourishment (PoU) in the world estimated to be in Africa. Beyond hunger, a growing number of people suffer from food insecurity which forces them to reduce the quantity and quality of their daily food, thereby risking malnutrition. Emergency aid policies can provide immediate, short-term assistance to the above categories; however, they can also lead to dependency on such aid while ignoring local sensory and cultural preferences or culinary traditions. Consequently, long-term solutions are indispensable to help local populations by strengthening their local food systems' resilience to shock. This can be achieved by adopting a multidisciplinary, multi-stakeholder approach, developing foods for low-income populations using country-specific raw materials with context-appropriate technology while satisfying nutritional, technological, cultural and environmental requirements and guaranteeing quality and safety.

With this in mind, this Ph.D. research project focused on improving the use of food resources in Africa by developing sustainable, highly nutritional food products using locally available, easily sourced raw materials and transforming these into food through the optimization of affordable technologies.

The thesis is divided in two sections (Figure 1). The first reports on an integrated multidisciplinary approach called "Pappa di Parma" which was used to develop, characterize and introduce novel, sustainable, energy-dense meals to treat moderate acute malnutrition. In addition, it reviews the scientific literature on novel Ready-to-Use Therapeutic Foods (RUTFs) including acceptability evaluations which focus on sustainability and local feasibility.

The second section describes a research programme which studied the effects of sprouting sorghum (*Sorghum bicolor* [L.] Moench) and subsequent drying treatments (40 °C for 12 h vs. 50 °C for 6 h) on the nutritional and technological features of derived flours used to develop sustainable and nutritionally enhanced flatbread for African countries.

Initially, the effect of sprouting and drying treatment on the starch and protein features of the derived flours was investigated, to determine whether different treatments would alter finished product functionality. Same effects on the micronutrient content, *in vitro* bioavailability, and antioxidant activity of the derived flours were also investigated. Subsequently, an insight into the molecular and rheological properties of sprouted sorghum flour-based dough provided through Low-Resolution Proton Nuclear Magnetic Resonance (LR ^{1}H NMR) and Dynamic Mechanical Analysis (DMA) techniques, together with the outcomes of previous findings, allowed to choose the flour obtained from sprouted sorghum dried at 40 °C for 12 h as the ingredient to be mixed with other locally sourced raw materials to produce composite flour. Then the functional features of sustainable composite flours, with a potentially high nutritional value, containing sprouted sorghum, tapioca, cowpea and wheat flours were studied. Lastly, optimized formulations of composite flours were used to develop economically sustainable, nutritionally enhanced flatbreads while preserving sensory acceptability and physicochemical properties.

The results demonstrated that the “Pappa di Parma” approach was a valid starting point to develop sustainable, bespoke alternatives to RUTFs in specific agricultural and socioeconomic contexts. In addition, optimizing the sprouting process by subsequent drying proved to be a sustainable, locally feasible method to improve the nutritional profile of sorghum flour. Moreover, the use of sprouted sorghum flour in composites was demonstrated to be an efficient strategy to promote the use of locally-available raw materials to develop technologically satisfactory, highly nutritional, and economically sustainable flatbread.

Overall, the optimization of local food-transformation/production technology is a sustainable, efficient approach to improve the use of local food resources while satisfying nutritional, technological, cultural, and environmental requirements, thus providing food solutions which can fruitfully be implemented in African cultural settings.

Keywords: Novel RUTFs, Pappa di Parma, Sorghum, Sprouting, Composite Flour, Flatbread

Sommario

Il pianeta non è sulla buona strada per raggiungere i “Global Nutrition Targets” fissati per il 2025, così come gli obiettivi di sviluppo sostenibile “Sustainable Development Goals (SDGs)” per il 2030. Dal 2014, il numero di persone che soffre la fame nel mondo è in costante aumento, e l’Africa possiede il più alto indice di denutrizione (prevalence of undernourishment, PoU). Inoltre, un numero crescente di persone vive in condizioni di costante insicurezza alimentare, che costringe a ridurre la quantità e la qualità di cibo consumato quotidianamente. I programmi di aiuto umanitario possono fornire assistenza immediata e a breve termine in contesti di emergenza, ma portano talvolta alla dipendenza da tali aiuti, che spesso risultano estranei alle tradizioni culturali e culinarie locali. Di conseguenza, diventa indispensabile offrire soluzioni a lungo termine in grado di rafforzare la resilienza dei sistemi alimentari dei paesi in via di sviluppo, in modo da valorizzare le produzioni locali e offrire i mezzi per poter affrontare le situazioni di necessità.

Tale obiettivo potrebbe essere perseguito tramite l’adozione di un approccio multidisciplinare che coinvolga più stakeholder, che miri allo sviluppo di alimenti mediante l’uso di ingredienti disponibili e tecnologie accessibili, soddisfacendo nel contempo i requisiti nutrizionali, tecnologici, culturali e ambientali, qualitativi e di sicurezza. Alla luce di ciò, il presente progetto di ricerca è stato finalizzato al miglioramento dell'utilizzo delle risorse alimentari di paesi africani, tramite lo sviluppo di prodotti nutrizionalmente bilanciati / arricchiti, usando materie prime localmente disponibili e tecnologie adeguate al contesto.

La presente tesi di dottorato è divisa in due sezioni (Figura 1). La prima sezione descrive l’approccio multidisciplinare chiamato "Pappa di Parma" utilizzato per sviluppare, caratterizzare e introdurre in Tanzania alimenti sostenibili ad alta densità energetica destinati a bambini di età compresa tra i 6 e 60 mesi di età, per il trattamento della malnutrizione acuta moderata. Contemporaneamente, la revisione della letteratura scientifica riguardante lo sviluppo e lo studio di accettabilità di nuovi Ready-to-Use Therapeutic Foods (RUTFs), ci ha permesso di metterne in luce aspetti chiave circa la loro sostenibilità e fattibilità.

Nella seconda sezione vengono riportati gli studi condotti sugli effetti della germinazione del sorgo (*Sorghum bicolor* [L.] Moench) e della seguente fase di essiccamento (40 °C per 12 h *vs.* 50 °C per 6 h) sulle caratteristiche nutrizionali e tecnologiche di farine derivate da impiegare nello sviluppo di pani piatti sostenibili e ad alto valore nutrizionale destinati a paesi Africani. In un primo lavoro, è stato studiato l'effetto della germinazione e del successivo trattamento di essiccamento sulle funzionalità di amido e proteine di farine derivate, in modo da determinare se diversi trattamenti possano influire sulla funzionalità del prodotto finito. Successivamente, ne sono stati studiati gli effetti sul contenuto in micronutrienti, sulla loro biodisponibilità *in vitro* e sull'attività antiossidante delle farine. In seguito allo studio delle proprietà molecolari e reologiche di impasti a base di farina di sorgo germinato tramite le tecniche di risonanza magnetica nucleare a bassa risoluzione (LR ^{1}H NMR) e di analisi dinamico meccanica (DMA), e in accordo con i risultati dei precedenti studi, è stata selezionata la farina da sorgo germinato ed essiccato a 40 °C per 12 h come ingrediente di farine composite in combinazione con altri sfarinati ottenuti da materie prime localmente disponibili. Pertanto, in uno studio successivo, sono state studiate le proprietà funzionali di farine composite sostenibili costituite da sorgo germinato, tapioca, farina di fagiolo dall’occhio nero e frumento. Infine, formulazioni ottimizzate di tali blend sono state usate per sviluppare pani piatti sostenibili e ad alto valore nutrizionale, preservandone l'accettabilità sensoriale e le proprietà fisico-chimiche.

I risultati del presente lavoro di ricerca hanno dimostrato che l'approccio "Pappa di Parma" è un valido punto di partenza per lo sviluppo di alternative sostenibili ai RUTFs commerciali. Inoltre, l'ottimizzazione del processo di germinazione mediante modulazione dell'essiccamento è un metodo sostenibile e accessibile per migliorare il profilo nutrizionale della farina di sorgo. L'uso di farina di sorgo germinata in farine composite è una strategia efficace per promuovere l'uso di materie prime locali per lo sviluppo di pani piatti di accettabile qualità, ad alto valore nutrizionale ed economicamente sostenibili. Nel complesso, l'ottimizzazione delle tecnologie di trasformazione/produzione è un approccio sostenibile per migliorare l'uso delle risorse alimentari locali, rispondendo altresì ad esigenze nutrizionali, tecnologiche, culturali e ambientali dei paesi Africani.

Parole chiave: Novel RUTFs, Pappa di Parma, sorgo, germinazione, farine composite, pane piatto.

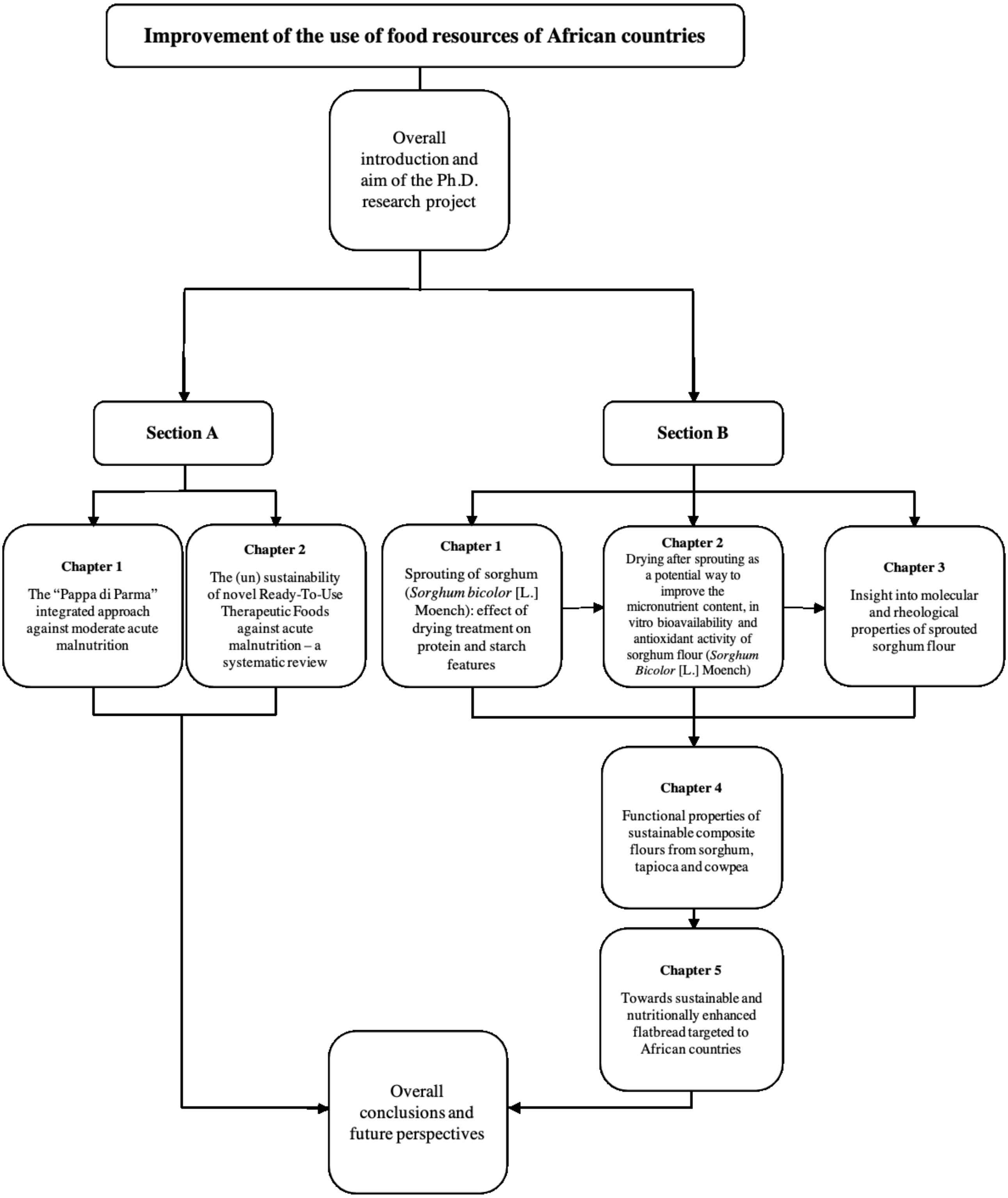

Fig 1. Workflow of the present Ph.D. thesis.

General introduction

Nearly 690 million people in the world have been estimated as undernourished (8.9% of the world population), with an increase of 10 million in just one year, 60 million since 2014, and the prospect of this number growing to over 840 million by 2030 (FAO, IFAD, UNICEF, WFP and WHO, 2020). The highest prevalence of undernourishment (PoU) in the world is estimated to be in Africa. In 2019, the African PoU figure was found to include 19.1% of the population, more than double the world average (8.9%) and corresponding to more than 250 million undernourished people (FAO, IFAD, UNICEF, WFP and WHO, 2020).

These estimates have revealed that the world is not on track to reach the Global Nutrition Targets set for 2025 (WHO, 2014) nor the Sustainable Development Goals (SDGs) set for 2030. In 2020, the most vulnerable population groups are also likely to see a nutritional profile that has worsened due to the health and socioeconomic effects of the COVID-19 emergency (FAO, IFAD, UNICEF, WFP and WHO, 2020), which is going to slow global efforts to achieve SDG 2 Zero Hunger by disrupting food systems and dramatically increasing hunger (HLPE, 2020). Preliminary assessments reveal that the COVID-19 pandemic is likely to increase the number of undernourished people by 132 million (FAO, IFAD, UNICEF, WFP and WHO, 2020), while the number of people living in extreme poverty may have grown by 100 million by the end of 2020 (World Bank, 2020).

Beyond hunger, a growing number of people are in a condition of food insecurity which forces them to reduce the quantity and quality of their daily food, thereby increasing the risk of malnutrition.

Deterioration in food security, especially for low-income communities, may be related to conflicts, climate shock, or economic downturns jeopardizing access to food for the more poverty-stricken. In 2019, two billion people in the world were estimated as not having constant access to safe, nutritious and sufficient food, of which 675 million were living in Africa (FAO, IFAD, UNICEF, WFP and WHO, 2020). It goes without saying that also short- and long-term adverse effects on food systems and on food security and nutrition caused by the COVID-19 pandemic are expected.

Emergency aid policies could provide immediate, short-term assistance to those locations affected by hunger and food-security related concerns; however, when implemented in prolonged emergency situations, they can lead to dependency on aid. Additionally, food aid might well not satisfy local sensory and cultural likings or food habits and could potentially destabilize local diets (Bounie et al., 2020).

An example of what said above, is provided by the development and introduction of Ready-to-Use Therapeutic Foods (RUTFs) to treat children affected by severe acute malnutrition. RUTFs are safe, palatable, energy-dense, lipid-based meals with a similar nutritional composition to therapeutic hospital diets used in severe acute malnutrition recovering, which were delivered from donor countries for home-based rehabilitation of wasted children.

The implementation of RUTFs in low-income communities undoubtedly changed for the better the approach used to treat children affected by wasting, empowering families to treat their children at home and encouraging a community-based approach (*Community Management of Acute Malnutrition*) over conventional hospital-based care. However, local communities have become totally dependent on this aid which, being industrially produced, is usually expensive, unsustainable for families, and also unfamiliar (Annan et al., 2015).

Consequently, aid policies endorsed by the global community and supported by scientific research and local national governments ought to be promoting sustainable self-reliance for vulnerable communities, so that they can achieve food security with adequate, safe and nutritious food over time. To this end, solutions need to be proposed that are effective not only in the short term, through the provision of any necessary food aid, but also in the long term, bringing value to local populations by strengthening local food systems' resilience to shocks to ensure the right to food (FAO, 2005), thus achieving SDG 2 (Bounie et al., 2020; HLPE, 2020).

The long-term objective can be reached by adopting a multidisciplinary, multi-stakeholder partnership approach, and to achieve this Food Science and Technology (FST) research could play a major role (Passino, 2016).

Accordingly, FST research applied to humanitarian actions was previously defined as "*the application of food science and technology to enhance food security, health, and economic prosperity for global humanitarian purposes*" (Bounie et al., 2020). In practice, the FST contribution in improving humanitarian food security aid could concern the design of fit-for-purpose foods which meet expectations on cultural appropriateness, convenience and quality, food quality and safety assurance through the whole humanitarian supply chain while promoting food wastes and losses reduction, and the strengthening of local food systems' resilience (Bounie et al., 2020). Examples of the key contribution provided by FST in humanitarian responses so far, are the following: the design of novel food aid with increased nutritional value and/or the improvement of the existing ones (Manary, 2006; Santini, Novellino, Armini, Ritieni, 2012); the attempts carried out to adapt food aid's taste to consumer preferences (Rakotosamimanana, & De Kock, 2020); the improvement of the nutritional value of Special Nutritious Foods along with their shelf-life extension through traditional technologies and plant-based ingredients and the increase in their convenience (Harvestplus, 2019; WFP, 2008; WFP, 2013; Bounie et al., 2020); the development and introduction in low-income communities of appropriate production processes and equipment, the implementation of the HACCP (Critical Control Points) methodology, of traceability systems and of rapid analysis methods for on-site quality assessments in order to increase the quality and safety of food (WFP, 2012; Bounie, 2018; WFP, 2019).

Hence, the crucial role of the FST in ensuring sufficient, safe and nutritious food to vulnerable communities in the long term consists in the development of foods by the use of country-specific raw materials with context-appropriate technologies while satisfying nutritional, technological, cultural, and environmental requirements and ensuring quality and safety (Bounie et al., 2020).

In this perspective, sorghum could represent a healthy food security crop for African countries and for this reason, its potential needs to be thoroughly investigated by FST researchers.

Cultivated mainly as food by low-income farmers, sorghum is a staple food for over 500 million poor and food-insecure populations in approximately 30 countries in the subtropical and semi-arid areas of the world (Hariprasanna & Rakshit, 2016).

Despite the potential beneficial effects on health related to the relatively low starch digestibility and the presence of health-promoting components of sorghum (e.g., dietary fibre, fat-soluble and B-vitamins, minerals, and polyphenols; Zhu, 2014), its particular physicochemical properties of proteins and starch make the use of this cereal for food production challenging.

First, sorghum prolamins (namely kafirins, which constitute from 77% to 82% of the proteins in the endosperm; De Mesa-Stonestreet et al., 2010) were proved to be more hydrophobic than corn, rice and wheat prolamins, thus limiting their interaction with water and their functionality in food products development (Teferra, & Awika, 2019). Another limiting factor for kafirins functionality is their structural organization in protein bodies: the three major kafirins subclasses identified according to molecular weight (α, β, and γ-kafirins) are organized in rigid spherical protein bodies in which the more protease-resistant and hydrophobic β and γ-kafirins surrounded the more digestible and less hydrophobic α-kafirin subunits. The high degree of polymerization and extensive disulphide bridges of protein structures, which become stronger upon cooking (Hamaker, & Bugusu, 2003), other than their strong interactions with tannins and starch, result in a 40–60% lower digestibility of kafirins in comparison to other cereals' prolamins and poor functionality when used in food applications (Teferra, & Awika, 2019). Moreover, the hydrophobic kafirin protein bodies surrounding starch granules limit the hydration of these latter, thus affecting starch gelatinization and digestion rate. Consequently, sorghum shows poor protein and starch functionality when used in food applications, both from a technological and nutritional point of view (Teferra, & Awika, 2019).

Besides, sprouting is a cost-effective biotechnological process traditionally used in sorghum-producing countries to overcome these concerns (Lemmens et al., 2019).

Sorghum sprouting has been used for centuries in the world to soften the kernel structure, to increase the nutritional value of this cereal, and to reduce antinutritional factors (Singh, Rehal, Kaur, & Jyot, 2017).

Sprouting promotes synthesis and activation of metabolic and enzymatic pathways in the grain, causing biochemical reactions which affect proteins, carbohydrates, lipids, vitamins, minerals, phenolic compounds and antinutrients, with an unavoidable impact on final product properties such as structure, bioactivity, flavour, stability, digestibility and technological functionality Singh, Rehal, Kaur, & Jyot, 2017.

From a nutritional point of view, the breakdown of proteins, carbohydrates and lipids into simpler forms, increases nutrient bioavailability and digestibility. Moreover, beneficial effects of sprouting could derive from the structural modification and synthesis of bioactive compounds such as vitamins, minerals and polyphenols, and from the increase in their availability thanks to antinutrients reduction (Afify, El-Beltagi, HAbd El-Salam, & Omran, 2011). Moreover, the biosynthesis and accumulation of compounds with high bioactivity enhances the antioxidant activity of products with potential positive health effects (Afify et al., 2011; Singh et al., 2017; Lemmens et al., 2019). The impact of sorghum sprouting on derived flour's protein and starch functionality has also been documented. Previous studies from literature reported an increase in solubility, water and oil holding capacity, foam and emulsion capacity and stability of flour obtained from sprouted sorghum, but worsened starch pasting behaviour and changes in textural properties of hydrolyzed starch compared to the flour from native sorghum (Abd Elmoneim & Bernhardt, 2010; Marengo, Bonomi, Marti, Pagani, Abd Elmoneim, & Iametti, 2015; Singh et al., 2017).

However, different effects of sprouting process on nutritional and technological features of sorghum may be related to variations in sprouting practices, such as soaking conditions, sprouting times and temperatures, and methods of drying of the sprouted grains (Singh et al., 2017; Lemmens et al., 2019).

Although sorghum sprouting phase in *sensu stricto* has been investigated in depth in literature, the effect of subsequent drying on the technological and nutritional functionalities of sorghum flour has been poorly explored. Given that the *temperature and time combination* is a key parameter in enzyme activity control (Muralikrishna, & Nirmala, 2005; Phiarais, Wijngaard, & Arendt, 2005), drying could modulate and prolong enzymatic activity during sprouting, conceivably affecting the flour's nutritional and technological properties.

Another potential sustainable strategy to strengthen food security while promoting resilience in developing countries could be the use of composite flour made from locally sourceable raw materials to produce foods.

The term composite flour defines a blend of flours obtained from various type of crops (e.g., tuber, legume, cereal and fruit flours), starches and other ingredients used in different percentage to totally or partially replace wheat flour in bakery and pastry products (Noorfarahzilah, Lee, Sharifudin, Mohd Fadzelly, & Hasmadi, 2014). The use of composites to produce foods is drawing much attention from research, especially due to the benefits that this practice entails for developing countries. The major advantages coming from this approach concern the reduction of the costs for importing wheat flour, the promotion of domestic agricultural production of native plant species, bolstering a better overall use of domestically grown raw materials while reducing post-harvest losses, and the increase in the provision of protein and biocomponents by the use of high nutritional value flours (Hugo, Rooney, & Taylor, 2000; Bugusu, Campanella, & Hamaker, 2001)

Moreover, preliminary research found in the literature demonstrated that, when used to produce bakery products, composite flours may guarantee a technological quality of the finished product overall similar to a product made with 100% wheat flour (Noorfarahzilah et al., 2014). However, the quality of derived food product is undoubtably related to the technological and nutritional properties of the added raw flours along with their percentage in the blend. Therefore, studies aimed at investigating the local feasibility of composites from locally available raw materials to be used as wheat flour alternatives, and at optimizing the formulation to improve the overall quality of derived finished products, should be encouraged.

In view of this, the use of composite flour for breadmaking could be a potential way to produce sustainable, nutritionally enhanced bread to be introduced into the daily diet in place of wheat-based one (Noorfarahzilah et al., 2014).

Overall, to intervene effectively and constructively in the fragile food systems in low-income countries, the first step is to investigate the needs and requirements of the target population, namely, the local community, as well as local food habits and traditions, available food resources and the technologies traditionally used to transform and process food (Bounie et al., 2020). Therefore, in order to propose a sustainable food alternative for families, it is necessary to study how to apply the technologies available to local food resources, to develop a food alternative which is better both nutritionally and qualitatively, but also acceptable from sensorial and cultural points of view. Furthermore, novel solutions need to be implemented locally through a training programme aimed at raising awareness and providing adequate information to the beneficiaries.

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Research purpose of the thesis

The general aim of this Ph.D. research project was to develop high nutritional value food products by means of accessible country-specific raw materials and their transformation into food through affordable technologies, in order to provide local-effective solutions to improve the use of food resources of African countries. Besides, this research aimed at optimizing locally feasible food-transformation/production techniques, in order to improve the nutritional and physico-chemical quality of food while decreasing post-harvest losses, thus delivering solutions that can successfully be implemented in African cultural settings.

Overall, the use of locally available ingredients and the optimization of domestic-feasible production technologies were intended to bring value to African communities by strengthening local food systems' resilience to shocks while ensuring the right to food.

In this perspective, two main topics were deepened, i.e., the development of novel Ready-to-Use Therapeutic Food (RUTFs) against children acute malnutrition, and the study of the drying treatment applied to sprouted sorghum (*Sorghum bicolor* [L.] Moench) as a potential sustainable process to modulate the derived flour's functionalities, in order to market finished products with an improved nutritional profile.

In this work, these two lines of research were presented in two different Sections, namely, respectively, Section A and Section B. The specific objectives of the studies carried out were the following.

<u>Section A</u>

- **Chapter 1**: the aim of the study reported in this chapter was to develop, improve and introduce "Pappa di Parma" as an alternative, innovative, sustainable, energy-dense meal against children moderate acute malnutrition in Tanzania. The meals were also studied for their shelf-stability and appropriateness to consumption under different storage conditions.
- **Chapter 2**: the work presented in this chapter was aimed at reviewing the scientific literature on the development novel RUTF and their acceptability evaluation, discussing key issues of sustainability and local feasibility.

<u>Section B</u>

- **Chapter 1**: the aim of the study presented in this chapter was to investigate the effect of different drying treatments (40 °C for 12 h *vs*. 50 °C for 6 h) applied to sprouted sorghum on the derived flours. Specifically, the functionality of starch and proteins were analysed to determine whether different drying treatments of the sprouted grain might modulate the flour functionality.
- **Chapter 2**: the research reported in this chapter attempted to evaluate the effect of drying treatments after sorghum sprouting on the micronutrient profile and antioxidant capacity of the derived flours.
- **Chapter 3**: the research presented in this chapter aimed at investigating the effect of sorghum sprouting and drying treatments on the derived flours' technological functionalities by means of Low-resolution Proton Nuclear Magnetic Resonance (LR ^{1}H NMR) and Dynamic Mechanical Analysis (DMA) techniques. A multivariate statistical approach was applied to assess differences among the molecular and viscoelastic properties of sprouted sorghum flour-water systems at different hydration levels in comparison to unsprouted sorghum and wholewheat flour doughs.

- **Chapter 4**: this chapter reported a research aimed at investigating the functional properties of sustainable composite flours with potential high nutritional value made by sprouted sorghum, tapioca, and cowpea flours for the production of sustainable and nutritionally enhanced bakery products targeted to African countries.
- **Chapter 5**: the study presented in this chapter aimed at developing nutritionally enhanced, economic sustainable and textural-quality acceptable flatbread formulations (made of sprouted sorghum, tapioca, cowpea and wheat flours) targeted to African countries. Formulations were also studied for their physico-chemical properties, some nutritional traits and sensory features.

Section A

Chapter 1

The "Pappa di Parma" integrated approach against moderate acute malnutrition

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Abstract

The use of Ready-to-Use Therapeutic Foods (RUTFs) is not a sustainable strategy to treat child moderate acute malnutrition due to its high cost and unfamiliarity. An integrated multidisciplinary approach called “Pappa di Parma” was used to develop, characterize and introduce alternative sustainable and energy-dense meals against malnutrition. Six formulations were developed by using basic accessible technologies and locally available ingredients (Tanzania), a daily portion of which meets RUTFs macronutrient requirements and most micronutrients RNI. Quality characterization, rheological properties and shelf-stability of formulae were assessed under different storage conditions disclosing the suitability and stability of no-water formulae in all tested storage conditions for almost under three months. Moreover, the cultural acceptance and the economical sustainability through the implementation in Tanzania were also evaluated. Overall, this study confirmed the “Pappa di Parma” approach as a valid starting point to sustainable alternatives to RUTFs, tailored to specific agricultural and socio-economic contexts.

Keywords

Sustainability, Infant malnutrition, Community engagement, Meal, Physico-chemical properties

Abbreviations

CMAM, community-management of acute malnutrition; RUTF, ready-to-use therapeutic food; SAM, severe acute malnutrition; MAM, moderate acute malnutrition; ASF, animal source food; RNI, recommended nutrient intakes; AI, adequate intakes; EFSA, European Food Safety Authority; RDA, recommended daily allowance.

1. Introduction

Malnutrition is one of the primary causes of child mortality according to the WHO (World Health Organization) (2019). Globally, in 2018, almost 200 million children under 5 suffered from stunting and/or wasting, and at least 340 million from vitamin and mineral deficiencies (UNICEF, 2019). Child malnutrition is extremely common in Middle and Eastern African states. In Tanzania alone nearly 40% of preschool children are malnourished (UNICEF, 2019), with 34% suffering from stunting and 6% from wasting. In combatting this dramatic burden, significant breakthroughs have been made in treating wasting.

The best-known approach is the *Community Management of Acute Malnutrition* (CMAM) that empowers families to treat acute malnutrition at home usually using specially formulated lipid-based foods in supplementary or complementary doses, namely Ready-To-Use Therapeutic Foods (RUTFs) (Gera, Pena-Rosas, Boy-Mena, & Sachdev, 2017; Lazzerini, Rubert, & Pani, 2013; Manary, 2006; UNICEF, 2019). RUTFs are safe, palatable, high-energy foods with appropriate amounts of nutrients, similar in composition to therapeutic hospital diets (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007). Since hospitalization is not always advantageous, especially in low-income rural scenarios, RUTFs frequently offer an alternative for home interventions. A household RUTF therapy provides about 200 kcal/kg of body weight/day proving efficacious in treating both Severe Acute Malnutrition (SAM) (Armini, Miele, Albero, Sacchi, & Cavella, 2018; Guimón and Guimón, 2012; Santini, Novellino, Armini, & Ritieni, 2013) and Moderate Acute Malnutrition (MAM), in appropriate dosages (Bharaniidharan & Reshmi, 2019; Briend et al., 2015; Gera et al., 2017).

However, RUTFs are not always appropriate to manage MAM, due to the high cost and unfamiliarity (Annan, Webb, & Brown, 2015). Indeed, the use of non-local ingredients and non-involving of the community are the main causes of RUTFs' failure among mothers of malnourished children (Ali et al., 2013; Dube et al., 2009; Guimón and Guimón, 2012; Vanelli et al., 2014). Nutritional education is vital to help mothers prepare meals (Ashworth & Ferguson, 2009; Vanelli et al., 2014), while satisfying children's sensory preferences is fundamental in nutrition interventions (Rakotosamimanana & De Kock, 2020).

Therefore, community engagement is vital in CMAM (Ali et al., 2013; Dube et al., 2009; Guimón and Guimón, 2012; Vanelli et al., 2014). Given the above, research has sought alternative foods which are potentially therapeutic against wasting, using ingredients accessible to families (Brixi, 2018; Collins & Henry, 2004; Dube et al., 2009; Lazzerini et al., 2013; Vanelli et al., 2014; Weber et al., 2017). A hand-made supplementary food known as "Pappa di Parma", prepared encouraging the active involvement of families and with local ingredients, proved effective against MAM in Sierra Leone (Vanelli et al., 2014). Nonetheless, the authors concluded that substituting milk powder and multivitamin mix with sustainable alternatives would enhance local feasibility. In this context, the aim of this work was to design new formulations to improve and tailor "Pappa di Parma" to specific agricultural and socio-economic contexts. In particular, "Pappa di Parma" integrated approach aimed to develop, improve and introduce "Pappa di Parma" as an alternative, innovative, sustainable, energy-dense meal against MAM in 6–60-month infants in Tanzania. The products' shelf-stability and consumption appropriateness were also assessed under different storage conditions.

2. Materials and methods

The experimental scheme was implemented in the village of Mvimwa (Rukwa Region, Tanzania), a poor area at 60 km from the city of Sumbawanga. In 2015, more than 600,000 Tanzanian children under 5 years of age suffered from acute malnutrition, of which 500,000 were moderate cases (UNICEF, 2020). At Mvimwa’s Benedictine Abbey, around 100 monks support local people by creating work, healthcare and education programmes.

2.1 *Formulae* development

2.1.1 Ingredient database

Firstly, a list of locally available ingredients was compiled from food composition tables from national agricultural reports (FAOSTAT) [Tanzania Food Composition Table (Lukmanji et al., 2008); West African Food Composition Table (FAO, 2012)], and communications collected in loco. The food/ingredients identified were grouped in different categories as follows: fruit and vegetables, legumes, starches (roots, tubers and cereals), seeds and nuts, fats and oils, dairy, meat-based ingredients, fish and others. Meats were excluded, since they are not commonly consumed being expensive, thus not widely accessible. Instead, fish was included being an accessible animal-source food (ASF) in low-income countries and a fundamental component of food-based strategies to fight nutrient deficiency (Roos, Wahab, Chamnan, & Thilsted, 2007). To complete the database of food locally available, nutritional information on each food/ingredient was obtained from local databases integrated with quantitative information from the Food Composition Table for Use in Africa, INFOODS/FAO (Woot-Tsuen, Busson, & Jardin, 1968) and the USDA, 2008.

2.1.2 Nutritional objectives

The nutritional composition of the food was defined with a number of nutritional and technological constraints to meet:

- A daily serving size equal to 200 kcal/kg/day calculated considering the energy requirements of malnourished children between 6 and 60 months (Ashworth, Khanum, Jackson, & Schofiel, 2003), in agreement with Vanelli et al. (2014).
- A nutritional composition in terms of percentage energy content from lipids and proteins per daily serving size established to meet the RUTF requirements of the joint statement from the WHO, WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007; Table 1). To avoid using industrial micronutrients preparations, when RUTF requirements could not be met, minimum tolerance values per day were used for micronutrients in line with Recommended Nutrient Intakes (RNI) specified by WHO and FAO, 2004. When the latter were not available, the Population Reference Intakes (PRI) or Adequate Intakes (AI) issued by EFSA, were used (EFSA, 2017). For these, they have been considered the values for 4- to 6-year-old children since they represent the highest available for our target population (6–60 months) (Table 1).
- The presence of carbohydrate-rich foods (cereals, roots and tubers), proteins from plants or animal-based staples (legumes, groundnuts, ASF) and lipids to increase energy concentration and workability and

palatability to the meal. Moreover, the presence of fruit and vegetables mainly for micronutrients was also searched for. Additionally, sugar was deemed mandatory to achieve the energy target and offer children a pleasant flavour. Besides nutrient and technological factors, safety-related features were also further constraints for meal formulation optimization.

Table 1. Energy and nutrient composition of Ready-to-use therapeutic food (RUTF) (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007) and minimum tolerance assumed for vitamins and minerals daily intake referred to 4–6 age child. The minimum tolerance was established considering the vitamin and mineral recommended nutrient intakes (RNI) specified by (WHO and FAO, 2004) and the adequate intakes (AI) released by the European Food Safety Authority (EFSA, 2017). RE = retinol equivalent; DFE = dietary folate equivalents; NE = niacin equivalents. a-TE: a-tocopherol.

	RUTF values (100 g)			Minimum tolerance values (per day)	
Component	Min	Max		WHO & FAO (2004)	EFSA (2017)
Energy	520	550	kcal	/	/
Proteins	10	12	% total energy	/	/
Lipids	45	60	% total energy	/	/
Sodium	/	290	mg	/	/
Potassium	1100	1400	mg	/	1100 mg/day
Calcium	300	600	mg	600 mg/day	
Phosphorus	300	600	mg	/	440 mg/day
Magnesium	80	140	mg	76 mg/day	
Iron	10	14	mg	6.3	
Zinc	11	14	mg	4.8	
Copper	1.4	1.8	mg	/	1 mg/day
Vitamin A (RE)	800	1100	µg	450 µg RE/day*	
Vitamin C	50	/	mg	30 mg/day	
Vitamin D	15	20	µg	5 µg/day	
α-tocopherol	20	/	mg	5 mg α-TE/day	
Thiamin	0.5	/	mg	0.6 mg/day	
Riboflavin	1.6	/	mg	0.6 mg/day	
Niacin (NE)	5	/	mg	8 mg NE/day	
Pantothenic acid	3	/	mg	3 mg/day	
Vitamin B6	0.6	/	mg	0.6 mg/day	
Folate (DFE)	200	/	µg	200 µg DFE/day	
Cobalamin	1.6	/	µg	1.2 µg/day	

* recommended safe intake

2.2 Benchtop production

Development and optimisation of several "Pappa di Parma" formulae were carried out at the Food and Drug Department laboratories of the University of Parma (Italy). Depending on availability, accessibility and affiliation with Italian customs, ingredients were purchased from Italian retail chains (CONAD), Italian organic stores (EcorNaturaSì Spa; Tibiona online store), or African grocery stores.

A benchtop “Pappa di Parma” production sought to replicate domestic food preparation in African countries. Preliminary experiments (>20) allowed to select the type and amount of ingredients, with appropriate pre-treatments and blending in order to obtain a homogeneous meal, pleasant for children in taste and texture, while meeting the pre-established nutritional requirements.

2.3 Rheological properties

A stress-controlled rheometer (MCR 102, Anton-Paar GmbH, Graz, Austria) with a parallel plate (25 mm diameter, depth 0.5 mm) based on the Peltier system with a solvent trap to avoid moisture loss, were used to measure viscosity and flow behaviour of different meals. For each test, a sample of approximately 4 mL was placed between the plates (2 mm gap) and allowed to relax for 30 s before analysis. Shear stress (τ) and apparent viscosity (η) were evaluated as a function of shear rate ($\dot{\gamma}$) from 0.1 to 18.5 s^{-1}. To analyse the effect of temperature on rheological behaviour and to simulate Sub-Saharan African climatic conditions, measurements were performed both at 25 °C and 40 °C. Commercial fruit-based (F) and meat-based (M) infant foods were taken as reference samples. Flow curves were fitted to the Herschel-Bulkley rheological model (Eq. 1) using SigmaPlot v. 12.5 software (Systat Software Inc., USA) to obtain rheological (τ_0, n and K) and statistical parameters (R^2) (Ahmed & Ramaswamy, 2006):

$$\tau = \tau_0 + K\,\dot{\gamma}^n \qquad [1]$$

Where τ_0 (Pa) is yield stress, K is the consistency index (Pa•s^n), and n is the flow behaviour index.

2.4 Physicochemical properties

After preparation, “Pappa di Parma” *formulae* were cooled at room temperature for 1 h and characterized for physicochemical properties (t0). The meals were divided into two main groups: (i) *water-formulae*, prepared with fresh ingredients or ones needing re-hydration, and (ii) *no-water formulae*, without added water. To investigate shelf-stability in a simulation of typical Tanzanian food production, *no-water formulae* were packed in polyethylene bags and stored for a period of 90 days (t90) under the following conditions (i) at 25 °C, vacuum-sealed, dark; (ii) at 25 °C, light; (iii) at 40 °C, light. The *formulae* were characterized at t0 (*water-formulae* and *no-water formulae*) and t90 (*no-water formulae*) for their moisture content [MC, g water/100 g sample; by weight loss after forced-air oven drying (ISCO NSV 9035, ISCO, Italy)], water activity [a_w; at 25 °C with Aqualab 4 TE (Decagon Devices, Inc., USA)] and pH (FiveEasy, Mettler-Toledo, LLC, USA). The pH of *no-water formulae* was measured by dispersing 1 g of sample in 6 mL of distilled water. The pH values were calculated using the Dilution Equation [Eq. 2]:

$$M1 * V1 = M2 * V2 \qquad [2]$$

where: H_3O^+ ions concentration before water suspension; *V1*: initial volume (mL); *M2*: H_3O^+ ions concentration after water suspension; *V2*: final volume (mL).

Colour of *formulae* was also measured using a CIELAB colorimeter (CM 2600d, Minolta Co., Japan) under standard illuminant D65. L^* (Lightness), a^* (degree of redness) and b^* (degree of yellowness) were measured using a 10° position of the standard observer. Ten determinations were taken for each sample at each storage time. Differences in colour between *no-water* samples during storage (t0 and t90) were evaluated using a ΔE value calculated as follows [Eq. 3]:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad [3]$$

All analyses were performed at least in triplicate for each storage period and condition.

2.5 Lipid oxidation

Measurement of lipid oxidation was performed after lipid extraction (EN ISO 659:2009, 2009). Peroxide value (PV) of *no-water formulae* was determined by iodometric titration (EEC Regulation 2568/91, 1991).

To verify the products' lipid oxidation during storage in the three aforementioned conditions, PV was measured over three months' storage (t0, t2, t7, t15, t50, t90). Three determinations were taken for each sample at each storage time.

2.6 Search for ingredients at local markets and preparation

In order to implement "Pappa di Parma" in Tanzania, a preliminary investigation of local availability, affordability and costs of the ingredients to prepare the meals was carried out at the most important trade centres of the closest city, Sumbawanga. Then, meals were prepared in the Mvimwa Abbey kitchen using basic available technology.

2.7 Satisfaction questionnaire

Meals were provided to 289 children (mean age ± SD: 22.1 ± 16.6 months, 48% females) from villages around Mvimwa Abbey. Children were recruited during medical visits at the dispensary by local monks and medical staff. The involvement of monks and medical personnel as trusted effective intermediaries encouraged the families' engagement and consensual participation in the survey. Moreover, their linguistic and healthcare mediation also ensured that the families received correct information and that the meals were safely administered to the children (Rakotosamimanana & De Kock, 2020).

After preparing the meals, families were recruited at the dispensary for a Satisfaction Interview. Firstly, monks used local language to explain objectives of the project and provide proper information about ingredients and meals preparation to the families. After tasting a half-spoonful of food, meal taste and acceptability was assessed by asking the children whether they liked it (closed Yes/No format) or, in the case of the youngest children unable to respond autonomously, by asking their mothers to interpret the children's perceptions. In addition, mothers were questioned about (i) their willingness to prepare "Pappa di Parma" by themselves, and (ii) their willingness to buy "Pappa di Parma" if available on the market, from a school or dispensary, using a closed Yes/No format.

2.8 Statistical analysis

A one-way variance analysis (ANOVA) and Duncan’s post hoc test were used to establish significant differences between the mean physicochemical values.

For each rheological parameter obtained from the data fitting, significant differences at 25 °C and 40 °C were assessed based on Student’s Distribution.

In addition, a Chi-square test was used to explore the association between categorical variables referring to satisfaction data. The statistical analysis was performed at the 0.05 significance level using SPSS statistical software (Version 25.0, IBM SPSS Inc., Armonk, New York, USA).

Values were expressed as mean ± standard deviation (SD) for continuous variables or as pure number and percentage in the case of categorical variables.

3. Results and discussion

3.1 Formulation and nutritional composition

Six candidate “Pappa di Parma” *formulae* were developed, varying the type and quantity of ingredients and/or proportions (Table 2). Three of them included water as *formula* WMP (water-milk power), WCB (water-corn-bean flour), WSB (water-sesame-bean flour). Other three included no water: NWP (no water-peanut flour), NWS (no water-soya flour), and NWF (no water-fish flour). All formulations contained palm oil (6.6–12.8%) which gave the product a creamy texture. *No-water formulae* also contained sunflower seed oil (8.9–12.5%) and sesame butter (15.0–17.9%) as a lipid phase, lending a more pleasant flavour. Milk powder (22.7%) and fish flour (12.5%) were the only two ASFs used. All “Pappa di Parma” *formulae* contained sugar (12.8–30.0%) to provide a sweet taste, while avocado (11.3–20.0%) and baobab pulp powder (7.5–10.3%) were the two fruits used.

Table 2. "Pappa di Parma" *formulae* (g/100 g). WMP: water-milk power formula; WCB: water-corn-bean flours; WSB: water-sesame-bean flour formula; NWP: no water-peanuts flour formula; NWS: no water-soya flour formula; NWF: no water-fish flour formula. n.a.: not available data.

Category	Ingredient	Cost	WMP	WCB	WSB	NWP	NWS	NWF
Fats and oils	Palm oil	1.31 $/L	9	8.5	6.6	8.9	12.8	12.5
	Sunflower seed oil	1.74 $/L	/	/	/	8.9	10.3	12.5
	Sesame butter	/	/	/	/	15.6	17.9	15
Seeds and nuts	Sunflower seed flour	/	/	/	/	/	/	10
	Sunflower seeds	0.87 $/Kg	16	/	/	/	/	/
	Peanuts	1.04 $/Kg	9	12.8	8.9	/	/	/
	Peanuts flour	n.a.	/	/	/	20	/	/
	Sesame seeds	1.74 $/Kg	/	/	11.1	15.5	/	/
Legumes	Bean flour	0.65 $/Kg	/	25.5	24.5	/	/	/

	Soya flour	1.74 $/Kg	/	/	/	/	25.6	/
Starchy foods and cereals	Cassava flour	1.74 $/Kg	16	8.5	11.1	/	/	/
	Corn flour	0.43 $/Kg	/	10.6	/	/	/	/
Animal-based products	Milk powder	11.31 $/Kg	22.7	/	/	/	/	/
	Fish flour	n.a.	/	/	/	/	/	12.5
Fruits and vegetables	Avocado	0.22 $/piece	11.3	21.3	20	/	/	/
	Baobab	1.74 $/Kg	/	/	/	8.9	10.3	7.5
Others	Sugar	1.22 $/Kg	16	12.8	17.8	22.2	23.1	30

The nutritional composition of the “Pappa di Parma” *formulae* is shown in Table 3 and S1.

Table 3. Nutritive values of “Pappa di Parma” formulae per 100 g meal. In brackets, the % covered of the recommended nutrient intakes (WHO and FAO, 2004) or of the adequate intakes (EFSA, 2017), referred to a daily serving size accounting for 2000 kcal. WMP: water-milk power formula; WCB: water-corn-bean flours; WSB: water-sesame-bean flour formula; NWP: no water-peanuts flour formula; NWS: no water-soya flour formula; NWF: no water-fish flour formula. Zero means no ingredient providing the nutrient.

Component (100 g)	WMP	WCB	WSB	NWP	NWS	NWF
Energy (kcal)	467.89	406.00	413.31	518.40	539.51	519.60
Proteins (% total energy)	10.80	12.13	11.93	11.13	9.96	11.66
Lipids (% total energy)	58.48	52.59	53.60	61.70	62.97	59.03
Sodium (mg)	101.61	7.17	4.33	4.07	5.77	41.63
Potassium (mg)	527.89 (+105)	303.39 (+36)	304.00 (+34)	270.76 (-5)	751.69 (+153)	303.80 (+6)
Calcium (mg)	247.82 (+77)	116.00 (-5)	211.20 (+70)	316.96 (+104)	225.21 (+39)	371.65 (+138)
Phosphorus (mg)	359.09 (+249)	216.28 (+142)	239.87 (+164)	219.39 (+92)	262.10 (+121)	346.65 (+203)
Magnesium (mg)	107.64 (+505)	59.51 (+286)	73.78 (+370)	115.00 (+484)	164.31 (+701)	109.85 (+456)
Iron (mg)	2.12 (+44)	9.62 (+652)	10.42 (+700)	7.22 (+342)	5.12 (+201)	4.00 (+144)
Zinc (mg)	2.76 (+146)	0.75 (-23)	1.32 (+33)	2.33 (+87)	2.21 (+70)	2.21 (+77)
Copper (mg)	0.36 (+54)	0.19 (-8)	0.59 (+184)	1.35 (+422)	0.83 (+206)	0.75 (+187)
Vitamin A (µg RAE)	551.18 (+424)	428.21 (+369)	336.40 (+262)	444.60 (+281)	672.31 (+454)	625.00 (+435)
Vitamin C (mg)	14.27 (+103)	9.91 (+63)	11.56 (+86)	17.87 (+130)	20.62 (+155)	15.21 (+95)
Vitamin D (µg)	0.00	0.00	0.00	0.00	0.00	0.25 (-94)
α-tocopherol (mg)	3.10 (+165)	3.96 (+290)	3.04 (+194)	4.41 (+240)	5.34 (+296)	5.71 (+340)
Thiamin (mg)	0.26 (+83)	0.19 (+57)	0.23 (+88)	0.26 (+69)	0.14 (-13)	0.36 (+133)
Riboflavin (mg)	0.41 (+193)	0.20 (+68)	0.18 (+49)	0.17 (+10)	0.34 (+109)	0.14 (-9)
Niacin (mg)	1.63 (-13)	2.06 (+27)	2.03 (+23)	8.18 (+295)	2.39 (+11)	3.00 (+44)
Pantothenic acid (mg)	1.02 (+45)	0.40 (-34)	0.41 (-33)	0.06 (-92)	0.06 (-93)	0.05 (-94)
Vitamin B6 (mg)	0.28 (+98)	0.17 (+38)	0.24 (+90)	0.26 (+66)	0.24 (+51)	0.25 (+59)
Folate (µg)	45.57 (-3)	30.49 (-25)	36.22 (-12)	31.80 (-39)	77.49 (+44)	41.68 (-20)
Cobalamin (µg)	0.68 (+143)	0.00	0.00	0.00	0.00	1.50 (+381)

Energy content varied between 406 and 539 kcal/100 g. Only NWS *formula* successfully achieved typical RUTF energy content, while NWP and NWF reached a value remarkably close to it (-1.6 kcal/100 g and -0.4

kcal/100 g, respectively). However, all “Pappa di Parma” *formulae* had an energy content providing 200 kcal/kg of body weight/day in line with WHO recommendations (Ashworth et al., 2003).

Out of the total energy content, 10.0–12.0% derived from proteins and 52.5–63.0% from lipids, in accordance with the optimal macronutrient composition of a RUTF (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007).

Similarly, the Na content of all *formulae* respected the maximum level set for RUTF composition. RUTF content values were also achieved for some minerals in certain preparations: Ca in NWP and NWF, P in WMP and NWF, Mg in WMP, NWP, NWS and NWF, and Fe in WSB.

However, it should be emphasised that RUTFs provide adequate nutrients for SAM subjects, while our targets were MAM children, for whom a standard RUTF can provide excess nutrients (De Pee & Bloem, 2009). By comparing the energy-parity formulations (2000 kcal) provided by a daily portion (satisfying the maximum energy requirement of 200 kcal/kg of body weight/day (Ashworth et al., 2003) for a 10 kg undernourished child), it was noted that the amount of P, Mg, Fe, and vitamins A, C, E and B6 was higher than the RNIs specified by WHO and FAO (WHO and FAO, 2004) and the AIs issued by EFSA (EFSA, 2017) in all six preparations. Instead, the amount of Ca, K, Zn, Cu, and vitamins B1, B2 and B3 was above the reference levels in five out of six *formulae*. Daily requirements of pantothenic acid (B5) were only covered by WMP, folate requirements by NWS. Cobalamin met the recommendations only in the *formulae* containing ASFs, i.e., WMP and NWF, as expected. As for vitamin D, the minimum value was not met by any of the “Pappa di Parma” *formulae* due to the absence or very low presence of ASFs. However, it is worth recalling that apart from the pre-formed vitamin provided by ASFs, vitamin D can be synthesised in the skin by exposure to sunlight. In the majority of countries lying around the equator, like Tanzania, endogenous synthesis may be the main source of this vitamin, with skin synthesis estimated to provide around 10 mg/day (WHO and FAO, 2004).

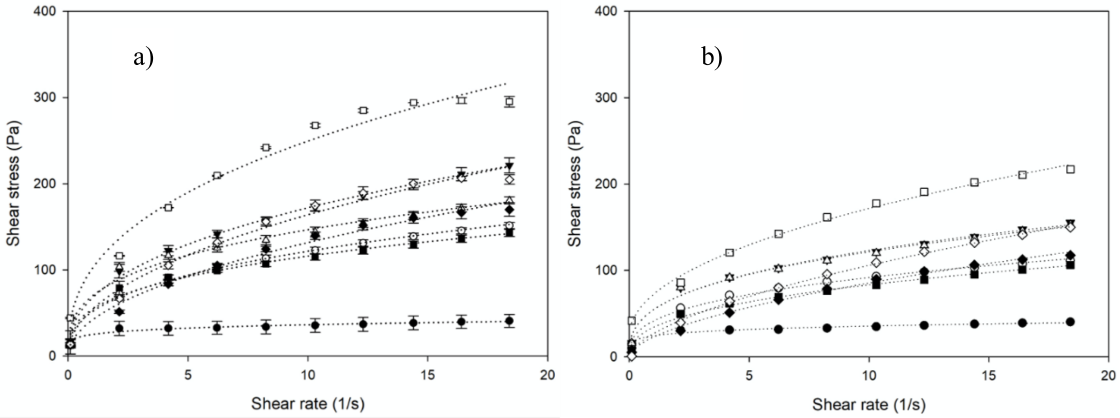
Although the amounts of certain nutrients provided by “Pappa di Parma” *formulae* were much higher than the RDA, we must recall that, during the design, the nutrient content was assumed not to vary consequent to the technological processes.

If this might be plausible (although not certain) for *no-water formulae*, it was presumed that a loss of some of the micronutrients of *water formulae* due to the ingredients processing during meal preparation may have occurred. Moreover, discrepancies which arose for some nutrients with respect to the reference values may be due to incompleteness of the reference nutritional databases and the need to respond to technological and sensory criteria using only accessible raw materials. Furthermore, criticalities also arose from the reduced inclusion of ASFs, which are fundamental sources of nutrients seldom consumed in low-income countries owing to cost or inaccessibility. Wherever necessary, the intake of certain micronutrients can be increased with small meals given throughout a child’s food day. It should be remembered, in fact, that “Pappa di Parma” is a specially formulated supplementary meal to be consumed at home as a part of children’s food day, which can include other food (e.g., breastmilk, fruit and vegetables) to complete nutritional needs.

Moreover, it is worth emphasizing that food fortification (e.g., wheat flour fortified with 0.02 mg/kg cobalamin, 2 mg/kg folic acid, 50 mg/kg zinc oxide and 38 mg/ kg sodium-iron EDTA commonly available at local Tanzanian markets) is a widespread basic alternative in low-income countries, complementing food-based approaches to satisfy people's nutritional needs (WHO and FAO, 2004).

3.2 Rheological characterization

Flow curves of "Pappa di Parma" *formulae* in comparison with commercial meat (M) and fruit (F) infant foods at 25 °C and 40 °C are plotted in Fig. 1.



[redacted]

Fig. 1. Flow curves measured by increasing shear rate at 25 °C (a) and 40 °C (b) fitted with the Herschel-Bulkley model. ● F food; ○ M food; ▼ WMP; △ WCB; ■ WSB; □ NWP; ◆ NWS; ◇ NWF; ····· Herschel-Bulkley model.

With the shear rate increase, the shear stress increased in a larger amount in all the meals tested indicating non-Newtonian pseudoplastic behaviour, while the presence of yield stress (τ_0) for most of the samples at 25 °C highlighted a plastic behaviour, in agreement with the literature (Ahmed & Ramaswamy, 2006; Alonso, Larrode, & Zapico, 1995; E. Alvarez, Cancela, & Maceiras, 2007; M. Alvarez, Canet, & Herranz, 2017; Krokida, Maroulis, & Saravacos, 2001).

The rheological behaviour was affected by temperature with a decrease in shear stress at 40 °C in all samples. Commercial F food exhibited the lowest shear stress values over all the shear rate range, NWP *formulae* had the highest ones, while M food and other "Pappa di Parma" meals showed an intermediate rheological behaviour (Fig. 1) at both the temperatures analyzed.

The Herschel–Bulkley model was used to describe the flow behaviour of the meals and the R2, τ_0, n and K parameters obtained have been summarized in Table 4.

Table 4. Herschel–Bulkley model parameters of "Pappa di Parma" *formulae* and industrial infant foods. For each sample, different lowercase letters indicate a significant difference (paired sample *t*-Test, $p \leq 0.05$) between temperatures for the same product. Different capital letters indicate significant differences (one-way ANOVA with Duncan's *post-hoc* test, $p \leq 0.05$) between different samples at the same temperature. WMP: water-milk powder formula; WCB: water- corn-bean flours; WSB: water-sesame-bean flour formula; NWP: no water-peanuts flour formula; NWS: no water-soya flour formula; NWF: no water-fish flour formula.

Sample	T (°C)	R^2	τ_0 (Pa)	K ($Pa \cdot s^n$)	n
F food	25	0.99	5.75±0.17 aC	18.20±6.87 aE	0.24±0.07 aE
	40	0.98	6.57±5.96 aC	16.93±9.25 aD	0.23±0.04 aD
M food	25	0.96	0.00±0.00 aC	73.14±2.53 aA	0.24±0.01 aE
	40	0.92	0.00±0.00 aC	59.71±0.11 bA	0.20±0.01 bD
WMP	25	1.00	0.00±0.00 aC	74.35±1.20 aA	0.37±0.01 aD
	40	1.00	0.00±0.00 aC	58.04±3.62 bA	0.34±0.02 aC
WCB	25	1.00	39.00±13.10 aA	26.40±4.33 bD	0.58±0.03 aA
	40	1.00	14.95±3.63 bC	56.72±3.94 aA	0.34±0.02 bC
WSB	25	1.00	24.03±0.07 aB	26.61±0.65 aD	0.52±0.01 aB
	40	0.99	14.95±2.57 bB	19.31±2.38 bCD	0.53±0.02 aB
NWP	25	0.97	28.70±0.70 aB	77.01±2.46 aA	0.44±0.01 aC
	40	1.00	25.99±9.28 aA	44.15±8.75 bB	0.50±0.05 aB
NWS	25	0.99	6.29±3.66 aC	36.56±2.48 aC	0.52±0.02 aB
	40	0.99	3.38±4.77 aC	20.19±2.14 bCD	0.59±0.04 aA
NWF	25	0.98	4.59±1.61 aC	50.57±2.41 aB	0.48±0.01 bBC
	40	1.00	0.00±0.00 bC	26.62±1.65 bC	0.58±0.02 aA

Yield stress (τ_0) represents a minimum shear stress to be exceeded before flow beginning and structure breakdown (Bourne, 2002; Guerrero & Alzamora, 1998) and is caused by strong interactions between the colloidal particles or the formation of links between the long-chain molecules which caused reticular structures to break (Alonso, Larrode, & Zapico, 1995). Taking in mind this, it is easy to understand the importance of this rheological parameter on the product's palatability. The F food exhibited plastic behaviour at both temperatures ($\tau_0 \approx 6$ Pa), while the M food showed no resistance to flow. Except for the WMP sample, all the *formulae* showed plastic behaviour with a τ_0 lower than 40 Pa (ranging between 4.5 and 39 Pa and 3–26 Pa at 25 °C and 40 °C, respectively). Overall, the parameter decreased with the temperature increase. Our τ_0 values were comparable to those found in the literature, where authors reported a wide range of variability depending on the baby food formulation and the temperatures of analysis [between 8 and 29 Pa for fruit and vegetable-based infant foods analyzed at 25 °C (Alonso, Larrode, & Zapico, 1995); up to 58 Pa for blueberry-puree based preparations analyzed in a temperature range of 27–93 °C (Kechinski et al., 2011); between 0.20 and 67 Pa for meat-based meat-based strained baby foods analyzed in a temperature range of 5–80 °C (Ahmed & Ramaswamy, 2007); between 0.54 and 1.76 Pa for sweet potato puree infant foods analyzed in a temperature range of 5–80 °C (Ahmed & Ramaswamy, 2006); between 2 and 14 Pa for vegetable – meat infant purees analyzed between 5 and 65 °C (Alvarez, Canet, & Herranz, 2017)].

The flow behaviour index (n) is a dimensionless number which indicates closeness to Newtonian flow behaviour (Bourne, 2002). Commercial and "Pappa di Parma" samples reported an n value ranging from 0.2 to 0.6 at any temperature, which is typical for products with pseudoplastic behaviour ($n < 1$), such as the infant foods previously investigated (Ahmed & Ramaswamy, 2006; Alvarez, Cancela, & Maceiras, 2007; Glicerina, Balestra, Pinnavaia, Dalla Rosa, & Romani, 2013; Krokida, Maroulis, & Saravacos, 2001). Moreover, the n value of almost all the meals was not significantly temperature - dependent. With regard to the consistency index (K, Table 5), F food samples showed the lowest K at both the temperatures considered (< 20 Pa•s^n), while M food ones showed high K index values (≈70 Pa•s^n and ≈60 Pa•s^n at 25 °C and 40 °C, respectively). Having an opposite consistency, the F and M foods were assumed as reference limits in the "Pappa di Parma" *formulae* K evaluation. Although large differences in the magnitudes of the K values were reported for the "Pappa di Parma" *formulae*, which ranged between ≈25 Pa•s^n and ≈77 Pa•s^n at 25 °C and between ≈20 Pa•s^n and ≈60 Pa•s^n at 40 °C, all the meals had intermediate behaviour among two commercial reference foods, thus demonstrating their rheological suitability for infant consumption. K decreased systematically with an increase in temperature, as expected and in agreement with previous observations on baby foods (Ahmed & Ramaswamy, 2006; Ahmed & Ramaswamy, 2007; Alvarez et al., 2007).

Rheological properties can be influenced by other factors such as the amount of solid vegetable fats and/or saturated fatty acids provided by the presence of ASFs (Glicerina, Balestra, Pinnavaia, Dalla Rosa, & Romani, 2013), the starch content derived from the use of pulse, cereal and tuber flours (Table 2) (Ahmed & Ramaswamy, 2006; Alonso, Larrode, & Zapico, 1995), the protein content and the degree of protein hydration (which are responsible for pseudo-gel structure formation in foods), the amount of water with its plasticizing and diluting effect. Moreover, the content and average size of the particles in the dispersed phase related to meals processing may have had an impact on rheological properties of different *formulae* (Alvarez et al., 2017).

3.3 Physicochemical properties

The physicochemical properties of the *water formulae* (t0) and the *no-water formulae* (t0 and t90) are reported in Table 5.

Table 5. "Pappa di Parma" physico-chemical characteristics measured at day 0 (t0) and 90 (t90) after production in different storage conditions. Values are expressed as means ± SD. WMP, WCB and WSB formulae require to be consumed immediately after preparation, thus data were acquired only at t0. For MC, a_w, pH, L^*, a^* and b^* at t0: different lowercase letters in each row indicate significant differences (one-way ANOVA with Duncan's post-hoc test $p \leq 0.05$) between samples at the same storage time. For each sample, different capital letters indicate significant differences (one-way ANOVA with Duncan's post-hoc test $p \leq 0.05$) between t0 and all the values recorded at t90 in different storage conditions.

		WMP	**WCB**	**WSB**	**NWP**			**NWS**			**NWF**		
	Day				*25 °C. vacuum. dark stored*	*25 °C. no vacuum. light stored*	*40 °C. no vacuum. light stored*	*25 °C. vacuum. dark stored*	*25 °C. no vacuum. light stored*	*40 °C. no vacuum. light stored*	*25 °C. vacuum. dark stored*	*25 °C. no vacuum. light stored*	*40 °C. no vacuum. light stored*
MC (%)	*0*	29.37 ± 0.95c	58.56 ± 0.3a	43.49 ± 0.14b		1.38 ± 0.12eA			2.96 ± 0.07dA			2.58 ± 0.08dA	
	90	/	/	/	1.31 ± 0.09A	1.27 ± 0.02A	0.77 ± 0.08B	2.72 ± 0.00B	2.60 ± 0.06B	1.58 ± 0.02C	2.51 ± 0.01A	2.32 ± 0.09B	1.94 ± 0.06C
a_w	*0*	0.90 ± 0.00c	0.99 ± 0.00a	0.97 ± 0.00b		0.41 ± 0.01eA			0.45 ± 0.00dA			0.39 ± 0.02eAB	
	90	/	/	/	0.38 ± 0.00A	0.35 ± 0.01B	0.30 ± 0.00C	0.38 ± 0.00B	0.36 ± 0.00C	0.32 ± 0.00D	0.41 ± 0.01A	0.36 ± 0.01B	0.31 ± 0.01C
pH	*0*	5.78 ± 0.08c	6.29 ± 0.1a	6.13 ± 0.10b		4.23 ± 0.06dA			4.14 ± 0.02eA			3.63 ± 0.07fC	
	90	/	/	/	3.99 ± 0.02bB	3.92 ± 0.04B	3.93 ± 0.04B	3.83 ± 0.05B	3.76 ± 0.03BC	3.78 ± 0.03B	3.75 ± 0.02A	3.71 ± 0.00AB	3.74 ± 0.04AB
L^*	*0*	56.2 ± 0.4c	65.64 ± 0.65a	58.65 ± 1.5b		51.09 ± 0.38dB			51.39 ± 0.37dC			44.77 ± 1.05eB	
	90	/	/	/	51.71 ± 0.76A	50.94 ± 0.63B	50.72 ± 0.55B	57.35± 0.74A	56.26 ± 0.53B	57.02 ± 0.42B	45.55 ± 0.83A	45.46 ± 0.59A	45.83 ± 0.85A
a^*	*0*	13.51 ± 0.48c	10.59 ± 0.35f	12.36 ± 0.91d		16.51 ± 0.41aBC			14.71 ± 0.16bD			11.6 ± 0.57eA	
	90	/	/	/	16.78 ± 0.58AB	16.89 ± 0.32A	16.38 ± 0.19C	17.60 ± 0.81A	17.1 ± 0.35B	15.7 ± 0.40C	10.94 ± 0.43B	10.00 ± 0.34C	9.71 ± 0.32C
b^*	*0*	48.08 ± 1.73b	50.2 ± 1.91a	47.25 ± 2.6b		39.27 ± 1.04dA			42.02 ± 0.84cD			30.18 ± 1.8eB	

	90	/	/	/	34.92 ± 1.92C	35.72 ± 1.13BC	36.26 ± 0.7B	49.36 ± 1.60A	48.06 ± 0.98B	46.48 ± 0.90C	31.23 ± 1.41A	28.46 ± 0.80C	28.73 ± 1.01C
ΔE	*0*	/	/	/	/			/			/		
	90	/	/	/	4.4	3.6	3.0	9.9	8.1	7.3	1.5	2.4	2.6
PV (mEQ/ kg oil)	*0*	/	/	/	0.98 ± 0.03b	0.98 ± 0.03e	0.98 ± 0.03d	0.98 ± 0.02c	0.98 ± 0.02c	0.98 ± 0.02c	0.98 ± 0.00de	0.98 ± 0.00d	0.98 ± 0.00d
	2	/	/	/	0.96 ± 0.00b	2.89 ± 0.14c	2.48 ± 0.38bcd	2.32 ± 0.49abc	4.07 ± 0.20b	2.74 ± 0.16a	2.33 ± 0.42bc	0.97 ± 0.00d	3.76 ± 0.07a
	7	/	/	/	2.78 ± 0.05a	5.42 ± 0.81a	2.86 ± 1.02bc	3.14 ± 0.58ab	8.42 ± 2.24a	2.72 ± 0.83a	2.85 ± 0.05b	8.72 ± 0.14a	3.36 ± 0.48ab
	15	/	/	/	2.41 ± 0.47a	4.18 ± 0.89b	1.85 ± 0.04cd	3.30 ± 1.53a	3.59 ± 0.99b	2.31 ± 0.51ab	4.81 ± 0.99a	3.37 ± 0.60b	3.19 ± 0.49b
	50	/	/	/	2.28 ± 0.57a	1.33 ± 0.54de	4.04 ± 1.32ab	2.02 ± 0.36abc	2.46 ± 0.76bc	2.08 ± 0.50ab	1.67 ± 0.08cd	2.59 ± 0.21c	1.70 ± 0.06c
	90	/	/	/	2.71 ± 0.22a	1.84 ± 0.03d	4.62 ± 1.22a	1.84 ± 0.01bc	0.85 ± 0.01c	1.69 ± 0.00bc	0.38 ± 0.38e	2.71 ± 0.26c	1.67 ± 0.14c

As expected, the moisture content of the *water formulae* (≈29.4%, ≈58.6% and ≈43.5% for WMP, WCB and WSB, respectively) at t0 was significantly higher ($p \leq 0.05$) than that of the *no-water formulae* (≈1.4%, ≈3% and ≈2.6% for NWP, NWS and NWF, respectively), due to the addition of water when preparing the meals (rehydration of beans, corn and manioc flours). Instead, the NWP, NWS and NWF *formulae* were all prepared without the use of water. In addition, after 90 days storage (t90), the MC (%) of the *no-water formulae* had slightly decrease contingent on the storage conditions (temperature, light, air exposure), especially when stored in a non-airtight bag at 40 °C and exposed to light (≈0.8%, ≈1.58%, ≈1.90% for NWP, NWS, NWF, respectively).

Water activity is one of the most critical factors in determining the quality and safety of foods, since it strongly impacts such reactions as enzymatic or non-enzymatic browning, lipid oxidation or microbial growth. As it is well known, the two critical a_w reference values at room temperature are 0.6 for limiting the growth of any microorganisms and 0.86 for the growth of pathogenic bacteria (Ross, 2007). As expected, the water activity (a_w) of all the *water formulae* was higher than 0.90 (0.90, 0.99, 0.97 for WMP, WCB, WSB, respectively), while the *no-water formulae* showed a_w values lower than 0.45 (0.41, 0.45, 0.39 for NWP, NWS, NWF), which significantly decreased ($p \leq 0.05$) after 90 days (t90) in all the storage conditions tested (Table 5).

The pH varied significantly ($p \leq 0.05$) among the samples resulting ≈ 6 for *water formulae* and ≈ 4 *for no-water formulae*. The *no-water formulae* acid pH value may have been influenced by the use of acidic baobab fruit pulp powder as an ingredient. At t90, the pH decreased in all the storage conditions for NWP and NWS, while a slight increase was recorded for NWF (Table 5).

As expected, the physico-chemical parameters recorded for *water formulae* were unable to ensure the shelf-stability of the products not immediately consumed. Furthermore, the lack of low temperature preservation technologies in low-income countries requires the consumption of meals immediately after preparation. In contrast, NWP, NWS and NWF meals included no water and the a_w and pH values can guarantee their stability towards microbial growth almost during the storage period tested.

Colour parameters (L^*, a^* and b^*) of the six "Pappa di Parma" meals measured at t0 and t90 are presented in Table 5.

The significantly higher L^* values recorded for the *water formulae* compared to those of the *no-water formulae*, were related to the diluting effect of the water which led to a less dense, light-coloured and therefore bright finished product. *No-water formulae* recorded significantly lower L^* and b^* values than *water formulae*, indicating a loss of colour lightness and of yellow hue, since the redness of the palm oil would have affected their colour more given the minimal moisture content. Overall, yellow –_red hues varied according to the formulation, type and amount of the ingredients. WCB recorded the highest L^* (≈65.6), the lowest a^* (≈10.6) and highest b^* values (≈50.2) related to its high MC (%), while the dark hues shown by the highest a^* measured for NWP (≈16.5) may be related to the reddish and greyish notes provided by palm oil and peanut flour. NWF was the sample with the lowest L^* (≈11.6) and b^* (≈30.2) values, since the addition of fish flour caused an evident browning of the finished product.

After 90 days' storage, colour parameters significantly changed in NWP, NWS and NWF, and ΔE values were calculated to evaluate whether these differences were perceptible to the human eye (the higher the value, the higher the perceived differences between t90 and t0 samples) (Limbo & Piergiovanni, 2006). ΔE values between 3.0 and 4.4 were found for NWP samples, leading to a perceptible colour difference from t0, while ΔE between 7.3 and 9.9 calculated for NWS denoted a strong difference in comparison to t0 for NWS samples. The colour of the NWF sample was more stable compared to the other samples after 90 days, showing a ΔE lower than 3 (noticeable difference) in all storage conditions. The changes of colour might be given by the outset of physico-chemical reactions attributable to factors related to the food composition (sugars, enzymes, metal ions, etc.) or to storage conditions (temperature, light, oxygen, etc.). Moreover, a possible slight phase separation between the oil and solid fractions may have accelerated these reactions, thus contributing to the products' colour change.

3.4 Lipid oxidation

Peroxide value (PV) is an estimation of the overall oxidation status for lipids and lipid-containing foods, especially in the primary phase of oxidation (Gray, 1978). It is a major parameter to evaluate the quality of lipids (Eldin, 2010) and its kinetic is often analyzed to determine the product's shelf life (Barden & Decker, 2016). The peroxide value results (PV) for the oil fraction extracted from *no-water formulae* at t0, t2, t7, t15, t50 and t90 over the three different storage conditions are shown in Table 5**.** At t0, the peroxide value is comparable between the different samples (≈0.98 mEq/kg oil); during the three months of analysis, the lipid oxidation followed variable kinetics contingent on the type of sample and the storage conditions. For the NWP sample, a slight increase in the PV up to t7 was observed when stored at 25 °C (≈2.8 mEq/kg oil and ≈5.4 mEq/kg oil under vacuum dark and light, respectively), while a slight progressive increase occurred up to t90 when stored at 40 °C (≈4.6 mEq/kg oil). The NWS and NWF samples showed similar oxidation kinetics under the same storage conditions, characterized by a slight significant increase followed by a progressive decrease. For both samples, the most protective storage conditions (vacuum, darkness, and 25 °C) delayed the PV peak to t15, a peak which was anticipated to t7 and t2 in the absence of a vacuum, the presence of light, and the highest storage temperature (Table 5).

In general, the slight fluctuation in the number of peroxides was never enough to exceed a value of 10 mEq/kg, thus remaining well below the threshold of unacceptability and showing good resistance to the oxidative process up to 90 days after production as a minimum. The results suggested that the various meals have a reasonably long shelf-life, and it is conceivable that deterioration of the product's quality concerning lipid oxidation only began after the observation period. These results can be justified by the water activity of the meals (≈0.4) and possibly to the plausible high presence of antioxidants provided by ingredients such as seeds and oilseeds, etc., that may have contributed to the oxidative stability of the meals.

3.5. Economic sustainability

An investigation of local availability and affordability of ingredients to reproduce the "Pappa di Parma" in local context was carried out at local markets closest to the city of Sumbawanga (Rukwa region, Tanzania). The investigation revealed the unavailability of high-protein peanut flour and fish flour, used in NWP and WNF meals, respectively, which, as a result, were not possible to be replicated in Tanzania. Despite ease in finding fresh fish at the markets thanks to the proximity to Lake Tanganyika, the lack of drying technologies suitable to obtain a safe product stable enough for human health prevented reproduction of NWF food in the Tanzanian context. These hindrances were highlighted only when "Pappa di Parma" was implemented at a local level. This suggests that to guarantee the feasibility of any type of nutrition interventions in an African country, a strong partnership driven by local context is mandatory.

Even so, NWF "Pappa", together with NWP, remain valid alternatives to supplement the diet of malnourished children in circumstances where there is availability of fishmeal or hyper-protein peanut flour.

All other ingredients were found with greater or lesser ease depending on the size of the market and the stock available. Ingredients such as beans, oilseeds and nuts, palm oil and starchy foods were the easiest to find since they represent the main energy sources of the local population's diet. The cost of the "Pappa di Parma" *formulae* was assessed taking into account the price of the raw materials and the quantities necessary for the production of a daily portion (Table 2). The cost calculated for a 100 g meal was \$0.37 for WMP, \$0.10 for WCB and WSB, \$0.19 for NWS. The higher price of the WMP formula is due to the presence of the powdered milk which, being imported, has a high price even though it is readily available at local markets. By and large, the "Pappa di Parma" was considered economically sustainable, in light of the disposable income of Tanzanian families (World Bank Group, 2019).

3.6. Satisfaction survey

The sensory quality of food contributes much to the emotional wellbeing of consumers and drives acceptability more than nutritional quality. Therefore, making sure that food products are culturally appropriate, acceptable and preferred is fundamental in nutrition intervention strategies (Rakotosamimanana & De Kock, 2020). Overall, the liking assessment revealed a high appreciation of all the preparations: 94% of children liked the WCB, 96% the WSB, 97% the WMP and 98% the NWS formula, with no association found between the "Pappa di Parma" *formulae* and liking.

Similarly, there was not an association between the Pappa di Parma" *formulae* and willingness to prepare them. The majority of the mothers (97% in the case of the WMP, 99% for NWS and 100% for WCB and WSB *formulae*) declared their willingness to prepare the "Pappa di Parma" by themselves for their child. When interviewed about their willingness to buy a ready-to-eat "Pappa di Parma" in a context of collective production, such as a school or dispensary, the majority of the mothers were in favour of buying WSB (96%), NWS (99%), and WCB (100%), while only 79% were in favour of buying the WMP formula, almost certainly because of the highest cost, showing an association between the Pappa di Parma" *formulae* and willingness to buy them ($p < 0.001$). In addition, irrespective to the preparation, no associations were observed between liking and sex of children and between liking and both willingness to prepare and willingness to buy the "Pappa di Parma". These results suggested that "Pappa di Parma" meals made with locally available ingredients are sustainable and culturally accepted alternatives which could be potentially effective against MAM.

4. Conclusions, recommendations and future perspectives

Six formulations of "Pappa di Parma" targeted to children aged 6 to 60 months affected by MAM were developed. In order to potentially deliver sustainable *formulae*, simple technologies and locally available ingredients (Rukwa region, Tanzania) were used. On the basis of the nutritional data, a daily portion of "Pappa" is potentially able to meet the energy and macronutrients requirements for malnourished children (Ashworth et al., 2003) and to provide a dose which is well above the recommended values for a healthy child for most micronutrients (EFSA, 2017; WHO and FAO, 2004). The implementation of the "Pappa di Parma" in local context confirmed that meals were found culturally accepted and economically sustainable, because of two key elements. Firstly, they were composed by locally available and economically accessible ingredients which are not aliened to eating habits and culinary traditions. Secondly, families (especially children and mothers) were involved in an education program. Furthermore, data collected from rheological, physico-chemical and lipid oxidation characterization proved the suitability and stability of *no-water formulae* in all storage conditions for three months.

All these features are certainly the strengths and the novelty of this study. However, consistent limitations that have to be analyzed to hypothesize future perspective, were found. Considering all *formulae* developed, from nutritional point of view, some limitations on the amount of certain micronutrients (e.g. vitamin B12) were ascertained. To address them, the integration of the home diet with fruits, vegetables, ASFs, and fortified ingredients, when possible, can be an effective solution to improve the nutritional intake of malnourished children in a domestic context using these preparations.

From safety and quality point of view, different issues can be considered based on the different type of "Pappa di Parma" *formulae* developed, that is *water* or *no-water formulae*. The *water formulae* require to be consumed immediately after preparation. Thus, their implementation is recommended in the framework of a structured educational intervention on the correct hygienic practices for food handling, transformation and consumption. Nevertheless, further researches are needed to verify micronutrient stability, especially when a pre-treatment of the ingredients is required.

For *no-water formulae,* the discussion should be addressed to the internationally accepted operating procedures [the Recommended International Code of Hygienic Practice for Foods for Infants and Children of the Codex Alimentarius (Codex Alimentarius Commission, 1981), the HACCP and the nationally adopted Bureau of Standards which regulate the production of food (Manary, 2006; Santini et al., 2013)] that specify a series of reference criteria (i.e. technological, microbiological and chemical) concerning ingredients and finished products that a RUTF is subjected to. Standard regulations established that RUTFs must appear as a smooth and uniform emulsion with small particle size (< 200 μm) to guarantee no grittiness and lumps and to avoid oil separation during the storage period (Manary, 2006; Santini et al., 2013). Moreover, despite the intrinsically microbiological shelf-stability of the products, the identification and limitation of potential microbial hazards must be considered (with the highest priority for *Salmonella*, *L. monocytogenes*, C. b*otulinum*) especially when ingredients and finished product are stored and handled under inappropriate conditions (Caron, 2012). The food should not contain any poisonous or deleterious substances, including antinutritional factors, heavy metals pesticides or mycotoxins in amounts that may represent a hazard to health (e.g., aflatoxin level 5 ppb maximum). The mycotoxins risk is especially connected to the non-safe conditions where locally foodstuffs are stored (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007). Thus, further investigations are needed to study and verify the conformity of our formulations to the RUTFs' production requirements, and to implement them in local production realities able to execute a quality control system on the ingredients, production lines and final products.

Moreover, an appropriately powered clinical trial is required to assess the effectiveness of "Pappa di Parma" meals to recover 6–60 months children from MAM, while an educational intervention program could be effective to scale up the approach to all families transferring knowledge and skills among mothers. Concluding, the "Pappa di Parma" *formulae,* composed by local available ingredients, and the integrated and multidisciplinary approach used represent a valid and solid starting point to further study the possibility to find economic and environmentally sustainable effective alternatives to RUTFs.

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Supplementary Material

Table S1. SFA, MUFA, PUFA and amino acid contents of "Pappa di Parma" *formulae* per 100 g meal.

Component (100 g)	WMP	WCB	WSB	NWP	NWS	NWF
SFA (g)	13.15	8.73	7.34	10.65	12.09	11.68
MUFA (g)	10.35	8.04	8.05	8.91	7.23	6.57
PUFA (g)	5.36	2.42	4.13	13.20	12.54	11.02
Tryptophan (mg)	246.93	159.34	144.73	181.41	182.36	199.32
Threonine (mg)	578.73	400.17	349.89	554.70	476.07	584.70
Isoleucine (mg)	704.64	430.43	373.05	566.59	520.59	643.75
Leucine (mg)	1171.91	827.77	665.57	1025.00	887.34	1069.91
Lysine (mg)	901.11	551.25	521.22	583.75	638.82	890.32
Methionine (mg)	254.21	152.69	132.98	252.39	205.11	377.75
Cysteine (mg)	179.65	135.65	103.20	209.09	190.84	212.32
Phenylalanine (mg)	698.15	568.40	480.20	773.50	577.57	626.20
Tyrosine (mg)	556.74	310.61	248.52	597.18	430.89	466.16
Valine (mg)	765.16	519.39	444.20	691.89	574.00	749.70
Arginine (mg)	887.85	771.21	664.02	1834.30	1052.59	1237.45
Histidine (mg)	363.12	279.52	232.27	390.05	304.00	396.09

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Chapter 2

Sustainability and acceptability of novel Ready-to-Use Therapeutic Foods in acute malnutrition management - A systematic review

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Author contribution: M.M.: conceptualization, manuscript writing.

Abstract:

Background: Despite being effective to treat acute malnutrition, "commercial" Ready-To-Use Therapeutic Foods (RUTFs) are not always appropriate in wasting management, being expensive and unfamiliar. Novel RUTFs have been developed meeting nutritional requirements while using local ingredients and/or technologies.

Scope and Approach: Systematically reviewing scientific literature on novel RUTF development and acceptability, discussing key issues of their sustainability.

Key Findings and conclusions: A total of 10 papers were included in this review, describing 18 novel formulations developed for African and Asian countries, while verifying cultural appropriateness and local acceptance. While much effort has addressed the nutritional, economic and qualitative aspects of novel RUTFs, research focusing on children's acceptance and cultural appropriateness of these meals is seriously lacking. 50% of the formulations resulted as equally acceptable as the standard RUTFs and only one formula was more appreciated. Community engagement and educational interventions are key elements in novel RUTF acceptance. Most novel RUTFs used locally for low-income populations are unsustainable because of unobtainable industrial ingredients (e.g., powdered milk and/or vitamin and mineral mix), use of not locally scalable production processes, or expensive. An innovative, multidisciplinary, multi-stakeholder approach is needed to develop "fit-for-the-purpose" RUTFs which satisfy consumer expectations in terms of taste, cultural appropriateness, acceptability, value, convenience, ease of production and safety, while satisfying nutritional requirements. With less dependency on food aid and greater reliance on local agriculture, such an approach can help food-insecure communities build resilience to acquire sufficient, safe, nutritious food in long-term *Community Management of Acute Malnutrition*.

Keywords: Sustainability, Infant malnutrition, Novel RUTFs, Acceptability, Locally produced RUTFs, supplemental feeding

Abbreviations: RUTF, Ready-To-Use Therapeutic Food; MAM, moderate acute malnutrition; SAM, severe acute malnutrition; CMAM, Community Management of Acute Malnutrition; PICOS, Population, Intervention, Comparison, Outcome, Study Design; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; LP, linear programming.

1. Introduction

Malnutrition appears as a triple burden which harms children's physical and cognitive health and their development. It occurs when the quantity of one or more macronutrients available to the body is inadequate to sustain optimal bodily functions (undernutrition), when the intake and absorption of micronutrients are too low for physical and cognitive development, immune-system and metabolic process functioning (hidden hunger), or when the intake of nutrients is oversupplied (overnutrition) (UNICEF, 2019).

Undernutrition in infants and young children manifests in two general forms: *stunting* and *wasting*. Stunting is a chronic disease resulting from poor nutrition in utero and in early childhood, which prevents children from attaining their fullest possible height and developing full cognitive potential (UNICEF/WHO/WBG 2020). Children are defined as stunted if their height-for-age is more than two standard deviations below the WHO Child Growth Standards median (WHO, 2019). Conversely, wasting is an acute, life-threatening result of poor nutrient intake and/or disease, manifesting as weakened immunity, susceptibility to long-term developmental delays, and an increasing risk of death (UNICEF/WHO/WBG 2020). Children are defined as wasted if their weight-for-height is more than two standard deviations below the WHO Child Growth Standards median (WHO, 2019). In addition, according to the severity, this acute strand of malnutrition can be classified as 'moderate acute malnutrition' (MAM) or 'severe acute malnutrition' (SAM).

Despite the actions taken worldwide to date to address this pervasive and corrosive burden (Fanzo et al., 2010), recent estimates have revealed still alarming malnutrition rates and insufficient progress to reach the *Global Nutrition Targets* set for 2025 (WHO, 2014) and the *Sustainable Development Goals* set for 2030. Indeed, globally in 2019, around 144 million children (21%) under 5 years old were estimated to be suffering from stunting, two thirds of them living in Africa and South-East Asia, while more than 47 million children (7%) were estimated as wasted (UNICEF/WHO/WBG 2020). Of particular concern are the COVID-19 pandemic's effects on the nutritional status of children living in low- to middle-income countries. The COVID-19 pandemic is expected to increase the risk of all forms of malnutrition, including wasting, mainly due to increased household food and income insecurity, in addition to changes in access to sanitation, water, healthcare facilities and protection services (Headey et al., 2020).

Prevention approaches, such as adequate maternal care during pregnancy and breastfeeding, access to healthy, nutritious and safe foods and water and sanitation services during childhood, along with approaches to address the causes of economic deprivation and inequity, are undoubtedly efficacious ways to eradicate undernutrition in the long term (UNICEF, 2019). Nonetheless, specific nutritional interventions can also mitigate the consequences of nutritional deficiencies in the interim (Schoonees Lombard, Musekiwa, Nel, & Volmink, 2019). SAM has ordinarily been treated in inpatient care with F-75, a milk-based therapeutic formula with a relatively low energy (75 kcal/314 kJ) and protein content (0.9 g per 100 mL) used during the first stabilization phase, and with F-100, a milk-based therapeutic diet, higher in energy (100 kcal/418 kJ) and protein (2.9 g per 100 mL) than F-75, being provided during the second rehabilitation phase to promote weight gain (WHO, 2013). SAM management restrictions on hospitals or therapeutic feeding centres, however, greatly limit its coverage and impact, since prolonged hospital care of wasted children is not always possible.

A community-based approach (*Community Management of Acute Malnutrition*, CMAM) has therefore been developed to treat wasted children in their local communities by providing ready-to-use therapeutic foods (RUTFs) or other nutrient-dense foods directly at home during rehabilitation. When properly combined with a facility-based approach for complicated SAM and implemented on a large scale, CMAM has proved efficacious in preventing the deaths of hundreds of thousands of children (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007).

RUTFs are safe, palatable, energy-dense foods with a similar composition to therapeutic hospital diets (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007), providing around 200 kcal/kg of body weight/day. In appropriate dosages, RUTFs have proved efficacious in treating both SAM (Guimón, & Guimón, 2012; Santini, Novellino, Armini, & Ritieni, 2013; Armini, Miele, Albero, Sacchi, & Cavella, 2018) and MAM (Briend et al., 2015; Gera, Pena-Rosas, Boy-Mena, & Sachdev, 2017; Bharaniidharan, & Reshmi, 2019). To date, the compressed biscuit BP100® (made by Compact, Denmark) and the oil-based paste Plumpy'Nut® (developed by IRD and Nutriset, France) are the two best-known RUTFs produced on a commercial scale (Collins, & Henry, 2004).

Despite their effectiveness in nutritional recovery from acute malnutrition, commercially produced RUTFs are not always considered appropriate in wasting management, principally due to their high cost and unfamiliarity (Annan, Webb, & Brown, 2015). Given that they are usually produced by donor countries and distributed by local governments, RUTFs can be expensive for low-income countries and their production is consequently limited. Furthermore, BP100® technology production is complex and expensive and therefore not transferable to small-scale local producers in developing countries; in contrast, the Plumpy'Nut® production process is simpler and easily transferable. However, the use of expensive ingredients not available locally in low-income countries (e.g., milk powder and industrial multivitamin mixes) decrease the Plumpy'Nut® recipe's suitability for widespread local production (Collins, & Henry, 2004). Furthermore, standard recipes for RUTFs typically include peanuts which is one of the food allergens most commonly associated with anaphylaxis in vulnerable children and are also known be easily prone to aflatoxin contamination, two factors which further limit their use (Collins, & Henry, 2004; Schoonees et al., 2019). In addition, industrially made RUTFs are considered alien to local traditions and are not always pleasant to children from a sensory point of view. The use of ingredients not available locally, a passive participation of the community, and children's dissatisfaction with the sensory properties of the meals have been reported as major causes of the RUTFs' lack of success among caregivers of undernourished children (Santini et al., 2013; Rakotosamimanana, & De Kock, 2020).

Based on the above, much research has tried to develop novel RUTF formulations which can meet the required nutritional values (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007) by using region-specific ingredients and/or locally available technologies, while satisfying children and their families' expectations in terms of convenience, taste, enjoyment and cultural appropriateness. This kind of approach could lead to less dependency on food aid and more reliance on local agricultural produce, thereby building resilience among local communities (Bounie et al., 2020).

While much effort has been put into addressing the nutritional, economic and qualitative aspects of alternative RUTF development, research specifically focusing on children's acceptance of these meals appears lacking, probably due to a widely shared belief that economically deprived consumers have no interest on sensory quality (Rakotosamimanana, & De Kock, 2020). The sensory quality of food contributes a great deal to consumers' emotional wellbeing and favours acceptability more than nutritional quality does. In addition, the reasons why consumers choose and accept certain foods in different countries and at different socioeconomic levels can vary considerably (Rakotosamimanana, & De Kock, 2020). Therefore, making sure that food products are culturally appropriate, acceptable, and preferred is fundamental, and when this key step is missing, the development process of novel RUTFs is incomplete.

The aim of this research is to review the scientific literature dealing with novel RUTFs development and acceptability evaluation, discussing strengths and weaknesses in terms of sustainability and local feasibility in light of the ingredients, production process, cost, sensory satisfaction, and cultural acceptability.

2. Materials and methods

2.1 Search strategy

A search for peer-reviewed articles was conducted to identify all the relevant research works published since January 2000. The initial search was conducted between July and August 2020. The PubMed search engine and the Scopus database were used to conduct the search, which was not restricted by language. The following keywords were used: (malnutrition) AND (child OR infant) AND (RUTF OR ready to use therapeutic food). The references in the eligible articles were then screened to find any additional relevant articles that might have been missed by PubMed and Scopus.

2.2 Data selection

The eligibility criteria were identified following the PICOS (Population, Intervention, Comparison, Outcome, Study Design) format. Inclusion criteria were the following:

- Population: children, malnourished children.
- Intervention: acceptability assessment of an alternative RUTF formulation made from locally available food ingredients, at least for the energy content equivalent to the standard RUTF.
- Comparison: industrially made RUTFs, traditional RUTF formula, Plumpy'Nut®.
- Outcome: alternative RUTF acceptability OR sensory qualities (AND price).
- Study design: original articles.

Exclusion criteria were the following:

- Population: hospitalized and/or HIV-positive children.
- Intervention: non-locally made RUTF, or locally made formulation not meeting the energy content of standard RUTF, inconclusive studies, medical and nutritional interventions.
- Outcome: any other outcome outside of the inclusion criteria.
- Study design: reviews and inconclusive studies.

The decision to include a study was based on title, abstract, and full-text screening.

The screening phase (title, abstract, and full-text) was carried out in duplicate by two different authors; a third author solved any conflicts that might have arisen in the screening phases.

3 Results

3.1 Search results

The literature selection process, in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) format, is shown in Fig. 1.

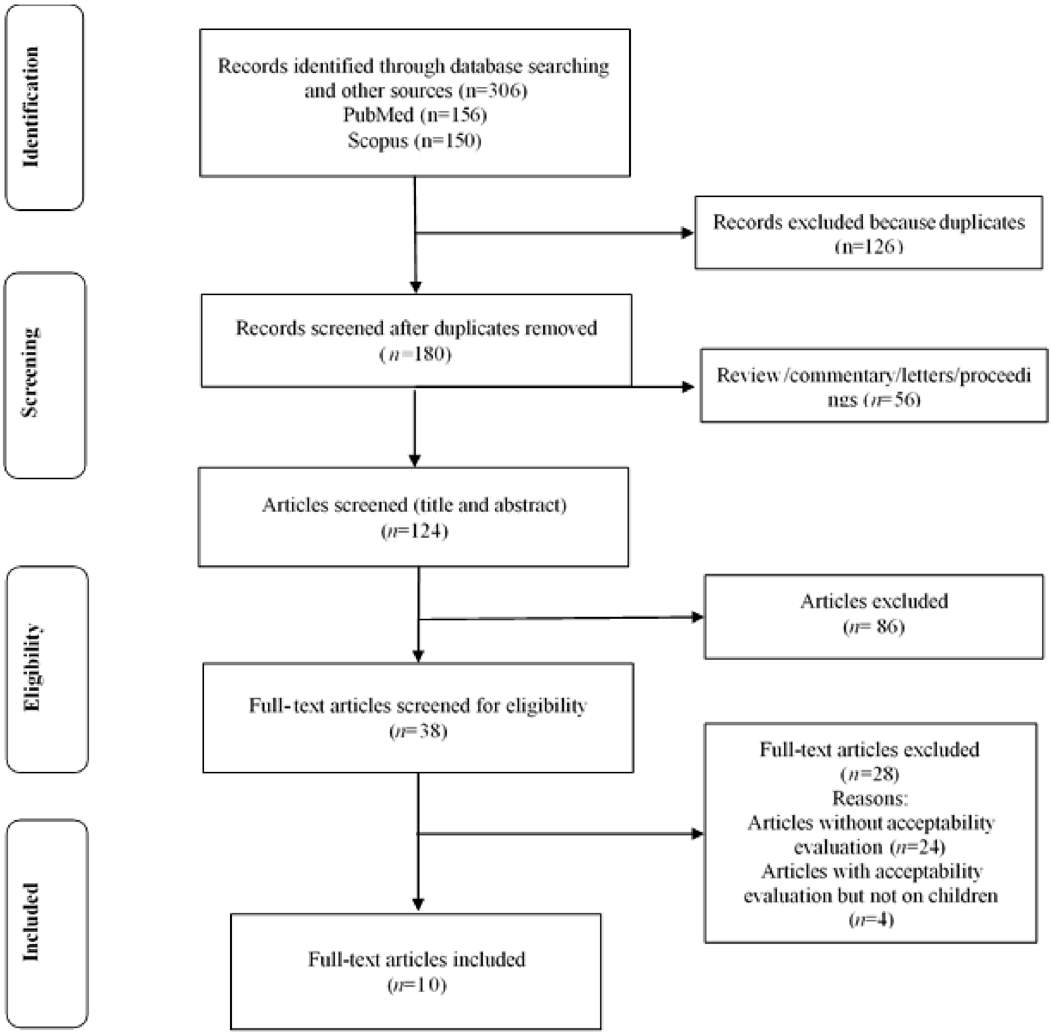

Fig. 1. Flow chart of the literature selection.

The initial search found 306 works. The articles were screened, and duplicates removed, resulting in 180 papers. Of these, 56 works were excluded because they were reviews, commentaries, letters, or proceedings, resulting in 124 articles. A further 86 articles were excluded on the basis of their title and abstract, along with 28 after a full-text analysis because they did not respect the inclusion criteria defined *a priori*. Among the latter group, two studies (Ali et al., 2013; Tadesse, Berhane, Hjern, Olsson, & Ekström, 2015) were excluded because they described an acceptability evaluation of standard RUTFs commonly used against SAM and not meals made with country-specific ingredients. Another study (Dube at al., 2009) was excluded because the newly designed meal did not meet the reference values for the energy content established for a standard RUTF (WHO, World Food Programme and United Nations, 2007). A study by Parlesak, Geelhoed, & Robertson (2014) was excluded because it referred to the economic and technological aspects of a basket of local foods and not a main meal. Other 4 papers were not included because the sensory analysis was carried out on adults (Miele et al., 2020) or was part of a linear programming model used for novel RUTF formulation, but not actually tested through an *in loco* food acceptability study (Dibari, Diop, Collins, & Seal, 2012; Ryan, Adams, Vosti, Ordiz, Cimo, & Manary, 2014; Weber & Callaghan, 2016). The remaining 20 articles describing alternative RUTFs did not include any food acceptability studies at all, thus not meeting the inclusion criteria. Among these, 12 dealt with the meal's nutritional/health effects (Oakley et al., 2010; Shewade, Patro, Bharti, Soundappan, Kaur, & Taneja, 2013; Bahwere et al., 2014; Kaleem, Aziz, Zaman, & Salimi, 2014; Vanelli et al., 2014; Irena et al., 2015, Jones at al., 2015; Bhandari et al., 2016; Bahwere et al., 2017; Sato et al., 2018; Sigh, Roos, Chamnan, Laillou, Prak, & Wieringa 2018; Jadhav, Karnik, Fernandes, Fernandes, Shah, & Manglani, 2019), 4 dealt with the meal's nutritional/health and economic assessments, this latter defined based on local prices (Singh, Kang, Ramachandran, Sarkar, Peter, & Bose, 2010; Akram, Suleman, & Hanif, 2016; Hendrixson et al., 2020) or on a general evaluation adapted to local market prices (Kohlmann et al., 2019), whereas 1 dealt with economic analysis only (Garg et al., 2018). Two other articles only characterized the novel meals technologically (Armini et al., 2018; Zuzarte, Mui, Ordiz, Weber, Ryan, Manary, 2020). Lastly, a study by Phuong et al. (2014) described the implementation phase of a novel RUTF but did not address the children's acceptance, nor nutritional, technological, or economic issues. Accordingly, a total of 10 articles met the inclusion criteria and were included in the present analysis.

3.2 Description of the included studies

Eighteen different recipes intended as alternatives to well-known standard RUTFs against SAM were proposed in the 10 articles included in the research. Table 1 summarizes the objectives of these studies, the methods, and the main information on the populations concerned, related to the 18 formulations described in the included papers.

Table 1. Description of selected original articles included in the review.

Population (country; children age (Y))	Children involved	N° of subjects enrolled	Aim of the study	Methods	Reference
Vietnam; 3-6Y	Healthy children with a WHZ-score between -3 and -1	67 (66*)	To develop and test the acceptability and the impact on anthropometry of a novel compressed bar RUTF	• Monitoring of children's meal intake (g) • Monitoring of children's reluctance to eat the meal • Evaluation of children's sensory acceptability by means of 3-point hedonic scale questionnaire with smileys • Evaluation of the impact on anthropometry	**Nga et al. (2013)**
Zambia; 4-11Y	Healthy Children	45	To develop and test the acceptability and tolerance of a novel RUTF *vs.* commercial RUTF	• Meal development by means of linear programming tool • Monitoring of children's meal intake (g) and illness frequency after meal consumption • Evaluation of children's acceptability by means of 5-point hedonic scale questionnaire	**Owino et al. (2014)**
Congo; <5Y	SAM Children	875	To compare novel RUTF efficacy in SAM treatment over commercial RUTF	• Evaluation of recovery rate, weight gain, LOS, hemoglobin, FM, body fat (%) and fat max index, FFM, FFMI, PhA, IM and AA plasma concentration • Monitoring of children's meal intake (g), acceptability and untoward effects	**Bahwere et al. (2016)**
India; <5Y	SAM Children	112	To compare novel RUTF acceptability and efficacy over "Defined Foods" used in SAM management	• Monitoring of children's meal intake (g) • Evaluation of untoward effects, weight gain, acceptability and rejection by means of structured questionnaire • Monitoring of children's facial expressions	**Thapa et al. (2017)**
Ethiopia; <2Y	MAM children	44	To develop and test the acceptability of a novel RUTF *vs.* commercial RUTF	• Meal development by means of linear programming tool • Monitoring of children's meal intake (g) • Evaluation of children's likeability perceived by caregivers by means of 5-point hedonic scale questionnaire with smileys • Evaluation of untoward effects after meal consumption	**Weber et al. (2017)** Meal 1
Ghana; <2Y		50			Meal 2
Pakistan; <2Y		51			Meal 3
India; <2Y		50			Meal 4
Bangladesh; <5Y	SAM Children	47 (30*)	To develop and test the acceptability of novel RUTFs *vs.* commercial RUTF	• Meal development by means of linear programming tool • Monitoring of children's meal intake (g) • Evaluation of children's acceptability perceived by caregivers by means of 7-point hedonic scale questionnaire with smileys	**Choudhury et al. (2018)** Meal 1 (Chickpea-based paste) Meal 2 (Rice-Lentil based paste)

Cambodia; 6 months-18Y	SAM Children	52 (and 57 caregivers)	To develop and test the acceptability of a fish based RUTF paste *vs.* commercial RUTF	• Evaluation of children and caregivers' acceptability by means of 5-point hedonic scale questionnaire with smileys and ranking test	**Sigh et al. (2018b)** Meal 1 (NumTrey-Paste)
Cambodia; <5Y		120	To test the efficacy and acceptability of fish based RUTF wafer *vs.* commercial RUTF	• Evaluation of weight gain • Evaluation of children's acceptability perceived by caregivers by means of 5-point hedonic scale questionnaire with smileys and ranking test	Meal 2 (NumTrey-RUTF)
Bangladesh; <5Y	SAM children	36	To develop and test the acceptability and the efficacy of a novel soy based RUTF *vs.* milk based RUTF	• Evaluation of eagerness to eat, meal intake (g) and monitoring of facial expression during meal consumption • Evaluation of untoward effects after meal consumption • Monitoring of changes in anthropometry	**Hossain et al. (2020)**
Tanzania; <5Y	MAM children	289	To develop and test the acceptability of "Pappa di Parma". The products' shelf-stability and consumption appropriateness were also assessed under different storage conditions	• Meal development by means of a nutritional database • Physico-chemical and rheological characterization • Evaluation of children and mothers' acceptability by means of closed Yes/No format questionnaire	**Marchini et al. (2020)** Meal 1 (WMP) Meal 2 (WCB) Meal 3 (WSB) Meal 5 (NWS)
Pakistan; <3Y	SAM Children	98	To test the effectiveness of energy-dense locally made food with micronutrients *vs.* commercial RUTF	• Evaluation of weight gain • Evaluation of caregivers' acceptability • Evaluation of untoward effects after meal consumption	**Muzaffar et al. (2020)**

*Number of children that completed the study.

- *Country of intervention*

The intervention settings are shown in Fig. 2.

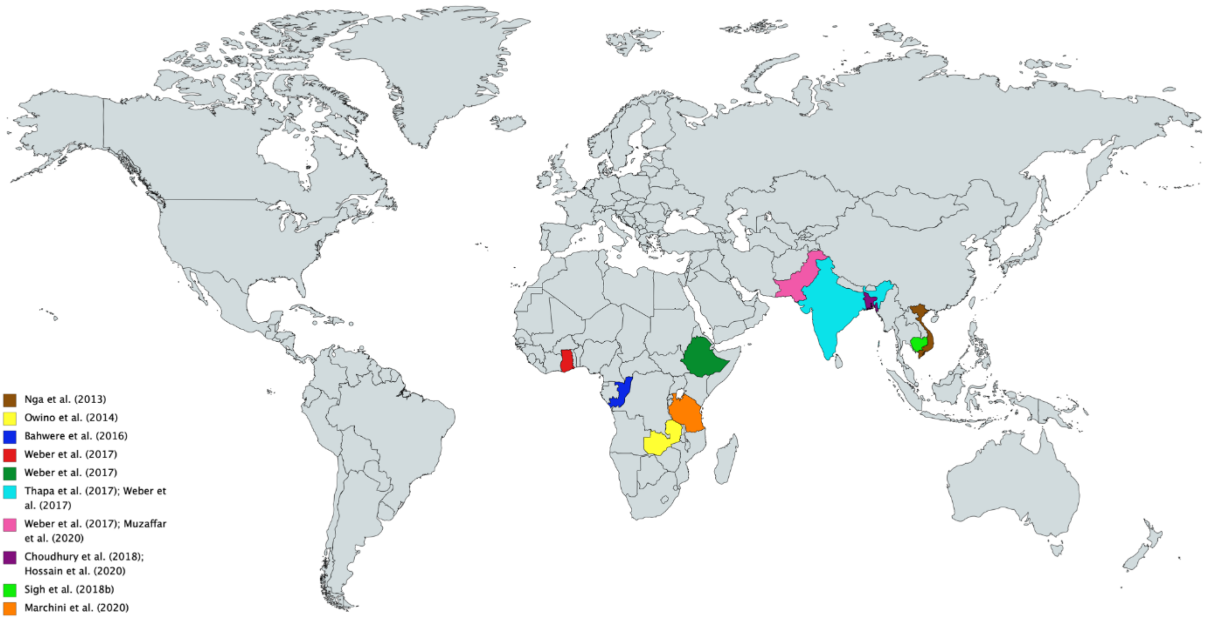

Fig. 2. Interventions setting around the world

Four meals were intended for malnourished children living in Tanzania (Marchini, Rosi, Giopp, Lolli, Scazzina, & Carini, 2020), two to be implemented in Cambodia (Sigh et al., 2018b), three in Bangladesh (Choudhury et al., 2018; Hossain, Huq, Islam, & Ahmed, 2020), two in Pakistan (Weber et al., 2017; Muzaffar, Shaikh, Chohan, Shah, & Ahmed, 2020) and two in India (Weber et al., 2017; Thapa, Goyal, Menon, & Sharma, 2017). Further States considered in the research were Vietnam (Nga et al., 2013), Zambia (Owino, Irena, Dibari, & Collins, 2014), Congo (Bahwere et al., 2016), Ethiopia and Ghana (Weber et al., 2017), for which only one recipe was developed.

- *Aim of the studies*

In accordance with the inclusion criteria of this research, all the studies were aimed at the development of novel RUTFs in children along with an evaluation of their cultural and sensory appropriateness. However, in addition to the food acceptability evaluation, 6 studies also monitored the appearance of side effects in the children after food intake (Owino et al., 2014; Bahwere et al., 2016; Thapa et al., 2017; Weber et al., 2017; Hossain et al., 2020; Muzaffar et al., 2020). Five investigations tested the novel RUTFs' effectiveness in malnutrition recovery by monitoring weight gain and increases in anthropometric measurements (Nga et al., 2013; Bahwere et al., 2016; Thapa et al., 2017; Sigh et al., 2018b; Muzaffar et al., 2020). A study by Marchini et al. (2020), assessed the physicochemical and rheological properties and shelf-stability of the meals to verify their suitability for consumption.

- *Study populations*

Almost all the studies which performed a food acceptability test as the only outcome enrolled a number of children ≤50 (Owino et al., 2014; Weber et al., 2017; Choudhury et al., 2018; Sigh et al., 2018b; Hossain et al., 2020), whereas the meals developed by Marchini et al., (2020) were evaluated by a greater number of children (289). In other cases, studies which monitored novel RUTF efficacy in SAM treatment while testing food acceptability enrolled a variable number of subjects, but always greater than 50 (Nga et al., 2013; Bahwere et al., 2016; Thapa et al., 2017; Sigh et al., 2018b; Hossain et al., 2020; Muzaffar et al., 2020).

Although RUTFs are commonly intended for children aged 6-60 months (Schoonees et al., 2019), three novel RUTFs were administered also to children > 5 years old (Nga et al., 2013; Owino et al., 2014; Sigh et al., 2018b). Moreover, only 6 studies actually enrolled children suffering from SAM (Bahwere et al., 2016; Thapa et al., 2017; Choudhury et al., 2018; Sigh et al., 2018b; Hossain et al., 2020; Muzaffar et al., 2020) mainly for ethical reasons. Other studies enrolled MAM children (Weber et al., 2017; Marchini et al., 2020), healthy children (Owino et al., 2014) or a mixed population composed by children with a WHZ-score between -3 and -1 (Nga et al., 2013).

3.3 Novel RUTFs formulations

Different approaches were used to design novel RUTFs targeted to specific socio-economic contexts.

Seven recipes were developed and optimized by using a linear programming (LP) tool (Owino et al., 2014; Weber et al., 2017; Choudhury et al., 2018). The LP tool was the most frequently applied operational research approach used to design sustainable novel RUTF formulations and to optimize their cost and palatability, while meeting the nutritional requirements. The LP method is based on a spreadsheet formulation tool which includes information on country-specific ingredients concerning nutritional composition, regional availability, food safety and quality, processing, and price (Dibari et al., 2012; Ryan et al., 2014). A simpler technique based on a database of country-specific raw materials completed with nutritional information for each food/ingredient was used by Marchini et al. (2020), while the USDA food composition database was used by Bahwere et al. (2016) for recipe formulation. Overall, four studies (Nga et al., 2013, Sigh et al., 2018b; Hossain et al., 2020; Marchini et al., 2020) selected ingredients while applying nutritional constraints to meet a standard RUTF composition. Two studies did not describe the methods and criteria used for the recipe formulation (Thapa et al., 2017; Muzaffar et al., 2020).

Recipes main ingredients are presented in Table 2.

Table 2. Evaluation of novel RUTFs sustainability based on ingredients, production process, cost and acceptability.

Formulation	**Absence of milk and/or peanuts**	**Use of industrial multivitamin mix (Yes/No)**	**Use of other industrially made ingredients**	**Costs and economic sustainability**	**Home feasibility (Yes/No reasons)**	**Acceptability**	**Reference**
Mung beans and soybeans-rice-sesame based compressed bar	Peanuts free	Yes	Milk powder Whey protein	Not evaluated	No (Ingredients and process)	Equally acceptable as commercial RUTF	**Nga et al. (2013)**
Soybean-maize-sorghum based paste (SMS-RUTF)	Milk and peanuts free	Yes	Palm olein and stearin	Not evaluated	No (Ingredients and process)	Equally acceptable as commercial RUTF	**Owino et al. (2014)**
Soy-maize-sorghum based paste (SMS-RUTF)	Milk and peanuts free	Yes	Palm stearin	Not evaluated	No (Ingredients)	Acceptable but less than commercial RUTF	**Bahwere et al. (2016)**
Peanuts and milk-powder based paste (Nutreal)	-	Yes	Milk powder	0.31$/packet Cheaper than "Defined Foods"	No (Ingredients)	Accepted by children and parents Well tolerated and more appreciated taste than Defined Foods	**Thapa et al. (2017)**
Peanuts-soybean-oat based paste	-	Yes	Acid whey Whey protein concentrate	1.25 $/kg	No (Ingredients and process)	Equally acceptable as commercial RUTF	**Weber et al. (2017)** Meal 1 (Ethiopia)
Soybean-maize based paste	Peanuts free		Whey protein concentrate	1.14 $/kg		Equally acceptable as commercial RUTF	Meal 2 (Ghana)
Lentil-almond-maize based paste	Peanuts free		Whey protein concentrate	1.45 $/kg		Less acceptable than commercial RUTF	Meal 3 (Pakistan)
Peanuts-lentil-oat based paste	-		Acid whey Whey protein concentrate	1.25 $/kg		Equally acceptable as commercial RUTF	Meal 4 (India)
Chickpea-based paste	Peanuts free	Yes	Dry skimmed milk; Soy lecithin	Not evaluated	No (Ingredients)	Equally acceptable as commercial RUTF	**Choudhury et al. (2018)** Meal 1
Rice-Lentil based paste						Equally acceptable as commercial RUTF	Meal 2

Fish-mung beans-rice-soybean-desiccated coconut-based paste	Milk and peanuts free	Yes	Maltodextrin	Not evaluated	No (Ingredients and process)	Less acceptable than commercial RUTF	**Sigh et al. (2018b)** Meal 1 (NumTrey-Paste)
RUTF wafer (rice flour- duck eggs-coconut meat) filled with RUTF paste						Equally acceptable as commercial RUTF	Meal 2 (NumTrey-RUTF)
Soy based paste (S-RUTF)	Milk and peanuts free	Yes	Soy protein isolate	Not evaluated	No (Ingredients)	Equally acceptable as milk based RUTF	**Hossain et al. (2020)**
Milk-peanuts-sunflower seeds-cassava-avocado paste	-	No	Milk powder	0.37 $/kg	Not fully (milk powder)	Acceptable	**Marchini et al. (2020)** Meal 1 (WMP)
Peanuts-bean-cassava-corn-avocado paste	Milk free		-	0.10 $/kg	Yes	Acceptable	Meal 2 (WCB)
Peanuts-sesame-beans-cassava-avocado paste	Milk free			0.10 $/kg		Acceptable	Meal 3 (WSB)
Sesame butter-soyabean-baobab paste	Milk and peanuts free			0.19 $/kg		Acceptable	Meal 5 (NWS)
Pulses based khichdi (rice)	Peanuts free	Yes	Milk powder	Not evaluated	No (Ingredients)	Accepted by mothers but less than industrial RUTF	**Muzaffar et al. (2020)**

Among the meals included in this review, four recipes included both peanuts and milk powder [Thapa et al., 2017; Marchini et al., 2020 (Meal 1)] similarly to commercial RUTFs, or peanuts and milk-derivatives [acid whey and whey protein concentrate, Weber et al., 2017 (Meals 1 and 4)]. Two other meals (Meals 2 and 3) proposed by Marchini et al. (2020) did not include milk, but tapioca, corn, beans, and avocado rather than peanuts as locally available ingredients in Tanzania. Besides, five formulae were produced without peanuts: the meal developed by Nga et al. (2014) was made from mung beans, soy, rice and sesame, while the two formulae proposed by Weber et al. (2017) included soybean and maize (Recipe 2, Ghana) or lentils, almonds and maize (Recipe 3, Pakistan) as plant-based ingredients. Recently, Choudhury et al., (2018) proposed a chickpea RUTF and a rice/lentil RUTF without peanuts. In contrast, five recipes did not include milk-based ingredients or peanuts at all: two of these contained soybean, maize and sorghum (Owino et al., 2014; Bahwere et al., 2016), one included soy protein isolates but no further details on the ingredients were provided (Hossain et al., 2020). Instead, Sigh et al. (2018b) used soy and mung beans, rice, and small indigenous fish to develop an RUTF paste (NumTrey-Paste), and a wafer filled with paste (NumTrey-RUTF) for the treatment of SAM; rice flour, duck eggs and coconut meat were used for wafer preparation. Last, the milk- and peanut-free formula (Meal 5) recently developed by Marchini et al. (2020) was prepared using sesame butter, soy, and baobab. Besides milk and peanut, vitamin and mineral mix powder was also used to meet the nutritional requirements for standard RUTF in the majority of the meals. Only in the study conducted by Marchini et al. (2020), the multivitamin mix was not used, with mainly fruit (e.g., avocado and baobab) being used as a source of micronutrients.

3.4 Acceptability of novel RUTFs

Different simultaneous approaches were used to assess the acceptability of the formulations and their appropriateness for consumption (Table 1). Product acceptability was mostly gauged by evaluating the amount of product consumed and/or untoward effects associated with eating it (Nga et al., 2013; Owino et al. 2014; Bahwere et al., 2016; Thapa et al., 2017; Weber et al., 2017; Choudhury et al., 2018; Hossain et al., 2020; Muzaffar et al., 2020). Monitoring nonverbal communication, like facial expressions or eagerness/reluctance to try the product have been other evaluations performed alternatively or in addition to previous assessments (Nga et al., 2013; Thapa et al., 2017; Hossain et al., 2020; Sigh et al., 2018b), being simple and immediate methods reflecting perception of a product and satisfaction with it. Moreover, to test product sensory acceptability and organoleptic qualities, researchers applied a simple closed Yes/No format questionnaire or standard hedonic test rating scales with either 3, 5, or 7 categories with smileys representing descriptors ranging from “like extremely” to “dislike extremely” or from “very bad” to “very good” (Nga et al., 2013; Owino et al. 2014; Thapa et al., 2017; Weber et al., 2017; Choudhury et al., 2018; Sigh et al., 2018b; Marchini et al., 2020). The preferential use of the pictorial smiley face scales, instead of the more complex and longer 9-point hedonic scale, may be justified by the low literacy levels expected among respondents.

In addition to children, the caregivers' acceptance was evaluated by Sigh et al. (2018b), Muzaffar et al. (2020) and Marchini et al. (2020). This latter study also evaluated the caregivers' willingness to pay and become involved in the preparation.

Acceptability evaluation results are resumed in Table 2. The consumer test performed by Marchini et al. (2020), was conducted solely on the newly designed formulations without using any commercial products as terms of comparison and revealed a high appreciation of the meals (≥ 94%). Instead, in the other studies, the formulations were compared with a commercial RUTF or a standard product commonly used in SAM management. Overall, 9 products resulted as equally acceptable as the standard [Nga et al., 2013; Owino et al., 2014; Weber et al., 2017 (Meals 1, 2 and 4); Choudhury et al., 2018; Sigh et al., 2018b (NumTrey-RUTF); Hossain et al., 2020]. The taste of Nutreal paste (Thapa et al., 2017) was more appreciated than the reference, whereas 4 further meals were less acceptable than the reference [Bahwere et al., 2016; Weber et al., 2017 (Meal 3); Sigh et al., 2018b (NumTrey-Paste); Muzaffar et al., 2020].

4. Discussion

In this systematic review, the potentiality of novel RUTFs, satisfying the standard RUTF nutritional requirements and accepted to both children and their families, was evaluated in terms of community sustainability.

In detail, the following issues of the novel selected RUTFs were considered: (i) the country-specific ingredients used; (ii) if the ingredients were available locally, (iii) the technological affordability and reproducibility of the meal in a local/domestic context, (iv) the economic sustainability of the ingredients and final products (v) the families' acceptance, including children's sensory preferences. Overall, a RUTF, to be sustainable, should include only country-specific, locally available, and economically accessible ingredients that are not alien to customary eating habits and culinary traditions. It should meet the pre-established nutritional requirements without including an industrial micronutrient mix, which is not affordable for low-income families. Additionally, it should seek to replicate domestic food preparation without resorting to industrial equipment and/or processes.

- *The sustainability of novel RUTFs intended as domestic feasibility and economic accessibility*

Standard RUTFs commonly consist of milk powder, vegetable oil, sugar, peanut paste, and a mixture of vitamins and minerals. Although milk sources are available worldwide, milk-based products are usually imported into low-income areas where cattle are difficult to breed. Peanut paste is normally industrially produced from peanuts toasted at high temperature. Minerals and vitamins are added to the recipe as an industrial powdered complex (Santini et al., 2013). As reviewed, novel RUTFs are commonly based on four ingredient categories: carbohydrate-rich foods (cereals, roots and tubers), protein from plants or animal-based staples (legumes, groundnuts, animal source foods), a source of energy (lipids, oil, sugar), and a source of mineral and vitamin (fruit, vegetables, fish or industrially made mixture). Sustainable RUTFs should not contain milk and peanuts in order to increase the economic sustainability and feasibility of local production, while reducing the risk of mycotoxin exposure coming from peanuts consumption (Santini et al., 2013). Indeed, with no need to import milk powder and/or peanuts, for producers in developing countries there would be a decrease in production costs. Moreover, the unsafe storage of imported/locally purchased peanuts to use in RUTF formulations may rise the aflatoxin contamination risk. Therefore, eliminating aflatoxin-contamination-prone peanuts may reduce the manufacturing challenges of producing a safe peanuts-based RUTF (Santini et al., 2013). Recipes in which milk powder and/or peanuts have been used should not be considered fully sustainable for families. On the contrary, recipes formulated without milk-based ingredients and/or peanuts should prove more sustainable compared to the previous ones. However, the weak point of most of the novel peanut-free recipes were the inclusion of industrially made ingredients, i.e., powdered milk and/or whey protein concentrate, as well as palm olein and stearin (Owino et al., 2014; Bahwere et al., 2016), maltodextrin (Sigh et al., 2018b), soy lecithin (Choudhury et al., 2018) or soy protein isolate (Hossain et al., 2020) in the other meals. Although less expensive than powdered milk (Weber, & Callaghan, 2016), whey is not an ingredient easily to find at local markets, and therefore its use did not increase the meals' local feasibility.

Moreover, other remarkable issue is the inclusion of powdered vitamin and mineral mix used in the majority of the formulae proposed in order to meet standard RUTF nutritional values (WHO, UNICEF, WFP and UN System Standing Committee on Nutrition, 2007). Since industrially made multivitamin mixes are usually imported and can be difficult to source at a local level, the price can vary a great deal depending on the market (UNICEF, 2009). The need to find substitutes for this ingredient is mandatory in the case of sustainable RUTF production. However, the target of each micronutrient established for RUTFs was sometimes not achieved (Marchini et al., 2020), despite the meals providing most micronutrients in a dose well above the recommended values for a healthy child (WHO and FAO, 2004; EFSA, 2017). However, it should be noted that, despite meeting the energy and macronutrient requirements established for RUTFs, the proposed formulae were specially formulated supplementary meals to be consumed at home as a part of MAM children's eating occasions, which can include other food (e.g., breastmilk, fruit and vegetables) to complete micronutrient needs.

The economic sustainability of novel RUTFs is closely related to the economic accessibility of the ingredients and processes used to deliver the meal. The employment of industrial equipment accounts for price increases and, in addition, a RUTF which necessarily needs sophisticated equipment would not be sustainable in a domestic environment for obvious reasons. Among the formulae mentioned in this work, most were assembled using industrial tools and processes, i.e., roasting of ingredients and/or industrial extrusion cooking (Nga et al., 2013; Owino et al., 2014; Bahwere et al., 2016; Weber et al., 2017; Sigh et al., 2018b) and the use of mixers and shakers to allow particle size reduction and homogeneous blending of the ingredients (Sigh et al., 2018b). These manufacturing steps are crucial for the product's safety and quality assurance. Indeed, roasting, cooking and high-temperature processing of ingredients are fundamental to reduce risks connected to the use of potentially contaminated raw materials, especially when handled and stored inappropriately. Besides, particle size reduction (<200 μm) and mixing procedures are functional to the stability of the emulsion, avoiding granulation and oil separation phenomena and increasing meal palatability and appropriateness to children's consumption (Santini et al., 2013).

Other technologies were used as strategies to improve the acceptability of the meal. A crispy elongated hollow wafer was filled with a fish based RUTF paste (Sigh et al., 2018b), which was produced with the following manufacturing steps: fish drying (with a greenhouse-type solar dryer for small-scale food industries), baking, grinding and extrusion. Additionally, an industrial compressor machine was employed by Nga et al. (2013) in order to compress the RUTF into a rectangular bar. On the other hand, Choudhury et al. (2018) provided only a generic description of the production steps required for the meal preparation (i.e., roasting, particle size reduction, blending), which did however appear easily transferable to a domestic level. Besides, all the meals designed by Marchini et al. (2020) were described as produced using only basic technology, and therefore easily transferable to local small-scale producers and domestic environments. No information on the meal production process was provided by three further studies (Thapa et al., 2017; Hossain et al., 2020; Muzaffar et al., 2020).

When locally produced, the overall cost of a RUTF is mainly down to the availability and price of the ingredients (Manary, 2006) and the degree of feasibility of the production process. As shown in Table 2, only three studies estimated the costs of the recipes developed (Thapa et al., 2017; Weber et al., 2017; Marchini et al., 2020), which were lower than those of standard RUTFs.

Three meals (Formulae 2, 3 and 5) developed by Marchini et al. (2020) were found the cheapest among those evaluable, if compared to commercial RUTFs [~3 $/kg (Santini et al., 2013)], which was to be expected since no type of industrially made ingredients were used, not even a multivitamin mix, and a simple production process was adopted. The slightly higher estimated price of Meal 1 (Marchini et al. 2020) and the meal developed by Thapa et al. (2017), could be explained by the use of powdered milk, while the costs estimated by Weber et al. (2017) reflected the use of industrial production process and ingredients.

Although the final price of many recipes was not detailed, it is reasonable to assume that the higher the use of country-specific, locally available ingredients and simple processes, the lower the final cost of the meal.

- *The sustainability of novel RUTFs intended as cultural and sensory acceptability*

Consumer studies give some insights into the development of acceptable food products (Rakotosamimanana, & De Kock, 2020), and are thus key aspects in developing and using novel RUTFs. With their wide range of cognitive abilities and attention spans, children need to be treated as a special population in any sensory or acceptability study, and a suitable testing environment and protocol must be adopted (Guinard, 2000). Sensory testing involving infants (0-18 months) and toddlers (18 months – 3 years) requires an indirect approach which mostly relies on non-verbal cues as bodily movement, vocalization, and facial expression, being not able to communicate verbally (Guinard, 2000).

Overall, “Acceptability” was defined as consumption of more than a certain target amount of the food offered (Nga et al., 2013; Owino et al., 2014; Choudhury et al., 2018) or, in the case of comparison with a commercial RUTF, “acceptable” indicated consumption of one product over the other (Thapa et al., 2017; Bahwere et al., 2016; Weber et al., 2017; Hossain et al., 2020). In addition, Thapa et al. (2017) categorized “product acceptance” as eagerly, not eagerly and poorly accepted based on the speed of consumption of the product and reactions as spitting, vomiting, crying, smacked lips, spillage, or grimaces. “Reluctance” to even try the meal was tagged by Choudhury et al. (2018) when children withdrew their head from the food, cried, kept their mouth shut or clenched their teeth, or became agitated, spat the food out, or refused to swallow it. Significantly, many different reactions and criteria were observed to evaluate children's satisfaction; establishing standard assessment criteria would prove extremely useful to standardize methodologies and findings when the adoption of non-verbal communication is unavoidable.

Sensory appeal and familiarity are two key factors affecting food choices (Rakotosamimanana, & De Kock, 2020). In addition to a more familiar usage and therefore preferred country-specific ingredients, strategies to improve the appeal and increase the pleasantness of a food in the eyes and mouth of children were sometimes used in the literature analysed, as anticipated in the previous paragraph. Indeed, the paste's texture and oil separation so unwelcome to children on a first evaluation, were improved by using a fine sieve in subsequent productions (Owino et al., 2014). Elsewhere, a visual appearance reminiscent of that of traditional local cuisine was adopted, e.g., the compressed bars and the two crispy elongated hollow wafers filled with RUTF paste developed by Nga et al. (2013) and Sigh et al. (2018b) for the children of Vietnam and Cambodia respectively. This strategy was overall successful since meals resulted pleasant to both children and their mothers. The use of sugar was also an axiom in the production of a novel RUTF, and where the list of ingredients is given, its use in abundance can be observed. However, it should be emphasized that in cases where the alternative RUTF was less appreciated than the comparison, these results might not be showing that novel RUTFs were unacceptable or would not be effective, but further investigations will be needed before their recommendation for nutritional interventions (Weber et al., 2017).

Reliability of consumer acceptance results is contingent with the extent to which the test panel actually reflects the opinions of a specific target consumer group (Rakotosamimanana, & De Kock, 2020). Many studies have included far too few respondents (<50, Table 1), and/or respondents belonging to an age group that is too narrow (Weber et al., 2017; Muzaffar et al., 2020) for the relevant consumer evaluation task to substantiate any dependable conclusions regarding the food acceptability of the items in question. However, most studies involved children belonging to a broad age range consistent with that for which the RUTFs are intended (6-60 months, Schoonees et al., 2019), which is assumed to have provided heterogeneous and acceptably representative information. In the studies conducted by Owino et al. (2014) and Sigh et al. (2018b), the acceptability test was extended to children up to 11 and even 18 years old, which may have provided inaccurate information given the documented rapid change in children's and teens' sensory preferences with advancing age (Guimard, 2000). Reliability of taste trials data may also be influenced by the overall serving size provided to children and how many times they tasted the meal. More reliable results may be expected from long-term acceptability trials (e.g., long-term period taste assessments or data collected during nutritional intervention trials) compared to short-term consumer acceptance data obtained with few liking assessments (Choudhury et al., 2018; Sigh et al., 2018b; Hossain et al., 2020; Marchini et al., 2020).

In low socioeconomic status communities, multi stakeholder partnerships and participatory approaches involving community workers (e.g., nursing and medical staff), community mobilizers, and healthcare operatives is key to access appropriate respondents and enhancing benefit (Bounie et al., 2020;

Rakotosamimanana, & De Kock, 2020). The local implementing partners most frequently involved were universities, international cooperation centres and hospitals, which in turn favoured mediation with paediatric departments, local healthcare centres, kindergartens, pre-schools and religious institutions, ensuring linguistic and healthcare mediation and therefore correct information circulation and safe administration of the meals. As anticipated, the role of the mothers also proved key in the acceptability studies, both as interpreters of their children's non-verbal communication and of the cultural acceptability of the formulations.

When informed of the ingredients and the usefulness of the proposed formulations, the mothers were generally enthusiastic about the products, willing to become involved in the preparation and purchase of it (Marchini et al., 2020), and to continue administering it even after the end of the study (Weber et al., 2017), believing that the products made their child stronger and healthier.

Collaboration with local institutions favoured the utilization of central locations for sensory analysis under trained personnel's control, which is to be recommended (Rakotosamimanana, & De Kock, 2020). The study by Weber et al. (2017) demonstrated that conducting tests at home should be avoided, even though a familiar and comfortable environment may seem beneficial for putting the children at ease. The authors reported that, although caregivers were instructed to offer the meal unadulterated to the child, the product was mixed with other foods, thereby inevitably affecting the children's willingness to consume the product as well as their appreciation.

5. Limitations, recommendations and future perspectives

Figure 2 highlights the limited number of countries where interventions have been carried out (coloured ones), if compared to many other countries which may need of interventions. This is of course the first remarks that has to be pointed out, which inevitably recalls the scientific community to study raw materials availability as well as cultural needs of many other countries that have currently not been considered yet.

Based on previous discussion, one of the key factors limiting the sustainability of novel country-specific meals against malnutrition is the difficulty of satisfying RUTF nutritional requirements without resorting to the use of industrial ingredients, above all, milk-powder and multivitamin mixes. While scientific research has proposed some plant-based alternatives to commercial ones that respect the composition in terms of macronutrients and energy (Table 2), the main obstacle is the difficulty in meeting micronutrient requirements without using a multivitamin mix, whose use has proved fundamental in the formulation of 15 out of 18 novel RUTFs, despite its unaffordability for local families. Marchini et al. (2020) demonstrated the feasibility of developing plant-based meals that theoretically (not proved on the final product) provide a dose of most micronutrients that is well above the recommended values for healthy children (EFSA, 2017; WHO and FAO, 2004) while meeting the energy and macronutrients requirements for malnourished ones. Additionally, *Arthrospira maxima* (Spirulina Algae) has been proposed by Miele et al. (2020) for a partial replacement of the multivitamin mix in RUTF formulations. Spirulina is a cell that can absorb and make bioavailable large amounts of minerals and vitamins, and its use has been proposed by the authors as sustainable for African peoples due to its his scalability and suitability for local weather conditions and its preparation which requires simple technology. However, a preliminary consumer trial on Italian adults revealed that products' sensory attributes were compromised by the addition of Spirulina Algae, and therefore further investigations are needed to improve the foods' sensory profile and to verify the products meet local people's sensory preferences and cultural acceptance. Furthermore, technological processes such as germination and fermentation, traditionally performed on a domestic scale in low-income countries, need to be evaluated as feasible, sustainable technologies to improve the nutritional values of plant-based ingredients (Singh, Rehal, Kaur, & Jyot, 2015; Erba et al., 2019; Erba et al., 2019; Lemmens et al., 2019). Interestingly, in this scenario, certain lactic acid bacteria have proved to be vitamin B12 producers and their addition to fermented dairy or soybean products should be investigated further to potentially increase the vitamin concentrations of such foods (Li, Gu, Yang, Yu, & Wang, 2017).

However, meeting the micronutrient requirements established for RUTFs by making exclusive use of sustainable plant-based ingredients remains a challenge today, especially as regards certain micronutrients supplied by animal-based ingredients.

While the inclusion of animal-based ingredients in novel RUTFs formulations is not always a viable path given their high cost and limited accessibility, on the other hand the use of fishery products should be urgently investigated. Fishery products are indeed an accessible and commonly consumed (mainly in the coastal area lands), animal source food containing protein, fat, vitamins and essential minerals and therefore fundamental components of food-based strategies to combat nutrient deficiencies (Roos, Wahab, Chamnan, & Thilsted, 2007). In the literature reviewed, only one study (Sigh et al. 2018b) performed an acceptability trials on children of a formulation containing fish obtaining encouraging results. In addition, other two studies (Ryan et al., 2014; Marchini et al., 2020) described a meal prepared with fish, but the acceptability of these novel RUTFs have not been tested. The use of fishery products for the development of food against child malnutrition must always ensure controlled correct conditions for handling and processing the raw material to ensure the products' microbiological and chemical safety (Ryan et al., 2014; Marchini et al., 2020). Studies of methods and ingredients to mask the unpleasant fishy taste and smell (Borg et al., 2019) may also be required.

Another fundamental aspect which must be addressed during RUTF development and before its administration is verification of its physico-chemical quality and safety. RUTF ingredients, production processes, and finished products must comply with the internationally accepted operating procedures established by the Codex Alimentarius Commission (Codex Alimentarius Commission, 1981), the HACCP, and the nationally adopted Bureau of Standards (Manary, 2006; Santini et al., 2013) as regards technological, microbiological and chemical reference criteria. Novel RUTFs production must therefore strictly adhere to these standards while verification of the products' physicochemical quality, stability and appropriateness to children's consumption is mandatory before use in consumer trials (Manary, 2006). Among the studies included in this research, 3 of them discussed the quality of the formulations by analytically verifying the products' compliance with nutritional, physicochemical and microbiological requirements (Owino et al., 2014; Weber et al., 2017; Choudhury et al., 2018). Besides, Sigh et al. (2018b) assessed the microbiological safety of NumTrey products, whereas Marchini et al. (2020) characterized meals from a physico-chemical and rheological point of view.

Considering that the full sustainability of a RUFT is reached when it may to be prepared at a domestic level, strategies to guarantee its safety and quality should be deepened. For example, lipid phase composition plays a major role in RUTF physico-chemical quality. If from one side the use of fats rich in polyunsaturated fatty acids may be a nutritional goal, from the other, they may cause rancidity in finished products being more susceptible to oxidative reactions. Therefore, if locally available, higher melting point lipids should be chosen in RUTF formulation to slow down oil separation during storage (Nestel et al., 2003). However, lipids melting at a temperature near to the body one should be preferred to improve the paste mouth-feed and facilitate swallow (Nestel et al., 2003). Besides, where feasible, the use of oats had been suggested in RUTF production, due to their ß–glucan antimicrobial properties and their potential to reduce oil separation (Hendrixson et al., 2020). In addition, the use of rich-in-antioxidants substances may contribute to meal stability (Nestel et al., 2003). In addition to ingredients, effort should be faced on the study of the effect of domestic processes on product quality and safety (e.g roasting and mixing/particle size reduction with mortar).

Overall, the implementation of formulations in developing countries therefore requires collaboration with local production companies and stakeholders located throughout the supply chain who are able to ensure the quality control of the product. In fact, food's safety and quality improvement is key in reducing hunger. High quality and safe food leads to better market access, reductions in food loss and waste, improved food security and fewer problems with foodborne diseases (Bounie et al., 2020). However, if implemented in a domestic context, the use of novel RUTFs is recommended as part of a structured educational intervention on correct hygiene practices in food handling, processing, and consumption, as well as nutrition counselling (Marchini et al., 2020).

Further research should cover all these aspects related to the sustainability and local acceptability of novel RUTFs prepared using locally available ingredients and technologies, testing also the efficacy of these new formulation to fight children malnutrition and increase awareness of the local populations.

6. Conclusions

This work systematically reviewed the scientific literature dealing with novel RUTFs development and acceptability evaluation, discussing key issues concerning sustainability and local feasibility.

The main outcomes emerged by the systematic review indicate that (i) too few studies have verified the cultural appropriateness and acceptance by local families of novel RUTF formulations targeted at a specific socioeconomic environment and that (ii) most of the novel country-specific RUTFs proposed cannot be considered *sustainable*, both due to the use of expensive, unobtainable industrial ingredients, or to the use of not locally scalable production processes, especially at a domestic level.

Additionally, consumer trials have revealed the necessity for community engagement and educational intervention in novel RUTF acceptance.

As a matter of fact, the research community is still far from making available sustainable RUFTs. Therefore, an innovative, multidisciplinary, multi-stakeholder partnership approach should be applied to develop "fit-for-the-purpose" RUTFs which satisfy consumer expectations in terms of taste and cultural appropriateness and acceptability, value, convenience, ease of production and safety, while satisfying nutritional requirements. Resulting in less dependency on food aid and a greater reliance on local agriculture, such an approach can help food-insecure communities to build resilience to acquire sufficient, safe, and nutritious food in long-term *Community Management of Acute Malnutrition.*

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Section B

Chapter 1

Sprouting of sorghum (*Sorghum bicolor* [L.] Moench): effect of drying treatment on protein and starch features

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Abstract

The nutritional and physicochemical properties of sorghum proteins and starch make the use of this cereal for food production challenging. Sprouting is a cost-effective technology to improve the nutritional and functional profile of grains. Two drying treatments were used after sorghum sprouting to investigate whether the drying phase could improve the protein and starch functionalities. Results showed that the drying treatment at lower temperature/longer time (40 °C for 12 h) extended the enzymatic activity that started during sprouting compared to the one performed at higher temperature/shorter time (50 °C for 6 h). An increased protein hydrolysis and water- and oil holding capacity were found in the flour obtained by the former treatment. Higher protein matrix hydrolysis caused high exposure of starch to enzymes, thus increasing its digestibility, while worsening the technological functionality. Overall, modulating drying conditions could represent a further way, in addition to sprouting, to improve sorghum flour's nutritional profile.

Keywords

Sprouting, drying, sorghum, kafirins, starch, physicochemical properties, functionality, nutritional profile

Abbreviations

US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; RH, relative humidity; TDF, Total Dietary Fibre; IDF, Insoluble Dietary Fibre; SDF, Soluble Dietary Fibre; RS, Resistant starch; DS, Digestible Starch; TS, Total Starch; BSA, bovine serum albumin; ΔH, enthalpy; T_{on}, onset temperature; T_p, peak temperature; T_{off}, offset temperature; S_p, swelling power; R/T, room temperature; SDS, sodium dodecyl sulfate; β-ME, β-mercaptoethanol; DH, degree of hydrolysis; UPLC, ultra-performance liquid chromatography; ESI-MS, electrospray ionization mass spectroscopy; SIR, Single Ion Recording; WHC, water holding capacity; OHC, oil holding capacity; BU, Brabender Units; LOQ, limit of quantification; ESEM, environmental scanning electron microscopy; DSC, differential scanning calorimetry; SDS-PAGE, sodium dodecyl sulphate - polyacrylamide gel electrophoresis; HMW, high-molecular weight; M_r, molecular weight; ANOVA, analysis of variance; AA, amino acids; EA, essential amino acids; NEA, non-essential amino acids; TA, total amino acids; EA/TA, ratio of essential amino acid (EA) to total amino acids (TA)

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal worldwide in terms of production and overall growing area (Hariprasanna & Rakshit, 2016). It is a key dryland food crop cultivated in marginal lands in more than 100 countries. Over 60% of global sorghum production (59.34 million metric tonnes, FAO 2018) comes from developing countries in Africa and Asia. Grown primarily for food by low-income farmers, it offers a staple for over 500 million poor and food-insecure people in around 30 countries in subtropical and semi-arid regions (Hariprasanna & Rakshit, 2016). Elsewhere, sorghum is mostly found in commercial farming for fodder and biofuel (Hariprasanna & Rakshit, 2016). However, its food use in developed countries is increasing, both as a gluten-free cereal for people affected by celiac disease, and for its nutritional potential which encourages industrial development of healthy alternative foods (Kulamarva, Sosle, & Vijaya Raghavan, 2009; De Mesa-Stonestreet, Alavi, & Bean, 2010; Zhu, 2014). Sorghum has huge potential for weight and obesity management due to the relatively low digestibility of its starch. Moreover, its other important nutrients include dietary fibre, fat-soluble and B-vitamins, minerals, and polyphenols (Zhu, 2014). In contrast, well-documented limitations on the food use of sorghum for food production are the poor nutritional and physicochemical properties of its proteins on cooking, which affects starch gelatinization and digestion rates (Zhang & Hamaker, 2005; De Mesa-Stonestreet et al., 2010; Zhu, 2014). These limitations are due to sorghum's protein organization; specifically, a high degree of polymerization, extensive disulphide bridges, and the high hydrophobicity of kafirins (representing 77% - 82% of the proteins in the endosperm; De Mesa-Stonestreet et al. (2010)) – as well as their encapsulation in protein bodies and their strong interaction with tannins and starch – make a challenge the use of this cereal for food production (Anyango, & Taylor, 2019).

Biotechnological processing (e.g., fermentation, germination) are used in sorghum-producing countries to overcome these issues (Singh, Sharma, & Singh, 2017; Lemmens et al., 2019). Moreover, these processes are widely used at household level in low-income countries. As regards germination, it is a proven sustainable approach which triggers synthesis and activation of intrinsic amylases and proteases, which in turn hydrolyse starch granules and proteins into simpler forms, increasing their *in vitro* digestibility and releasing free sugars and amino acids (Correia, Nunes, Barros, & Delgadillo, 2008; Afify, El-Beltagi, Abd El-Salam, & Omran, 2012). An impact on the flour's functional properties has also been documented: protein hydrolysis results in higher solubility, water and oil holding capacity, foam and emulsion capacity and stability than in native sorghum flour (Elkhalifa, & Bernhardt, 2010; Singh et al., 2017). Conversely, worse pasting behaviour and changes in textural properties of hydrolysed starch have also been reported (Marengo, Bonomi, Marti, Pagani, Elkhalifa, & Iametti, 2015).

Although sorghum sprouting has long been investigated, the effect of subsequent drying on the technological and nutritional functionalities of sorghum flour has been poorly explored so far.

Since the temperature and time combination is a key parameter in enzymatic activity control (Muralikrishna, & Nirmala, 2005; Phiarais, Wijngaard, & Arendt, 2005), drying could differently affect and prolong the enzymatic activities during sprouting, conceivably affecting the flour's nutritional and technological properties.

In the literature, many of the scientific works investigating the issue of germination applied to cereals have carried out a post-germination drying treatment at a temperature between 40 and 50 °C (Elkhalifa, & Bernhardt, 2010; Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011; Afify, El-Beltagi, Abd El-Salam, & Omran, 2011; Marengo et al., 2015; Nkama, Gbenyi, & Hamaker, 2015; Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017; Singh et al., 2017). While on the one hand a drying temperature of 50 °C may be more representative of the conditions adopted in industrial processes performed under controlled conditions of time, temperature and humidity (Nkama et al., 2015; Marti et al., 2017), on the other, a drying temperature of 40 °C is more representative of the conditions adopted in those contexts where germination is carried out at home and the sprouts are dried in the sun (Elkhalifa, & Bernhardt, 2010, Phattanakulkaewmorie et al., 2011). To assess the properties of sorghum flour obtained by sustainable but empirical processes could be of great relevance for the development of sorghum-based food products. Indeed, the outcomes of this research could contribute to market finished products with a tailored and potentially improved nutritional profile, especially for low-income countries where sorghum represents a staple food.

Therefore, this work investigated the effect of drying treatment conducted at 40 °C on sprouted sorghum, in comparison with a drying treatment performed at the higher temperature (50 °C). Specifically, the functionality of starch and proteins were considered to determine whether different drying treatments of the grain might modulate the product functionality.

2. Materials and methods

2.1 Sample preparation

Commercial white sorghum (*Sorghum bicolor* [L.] Moench) kernels were sprouted at an industrial sprouting plant (Bühler AG, Uzwil, Switzerland) under controlled temperature and humidity. The schematic representation of sample preparation is shown in Fig. S1. Sorghum was soaked in water (kernel:water ratio of 1:2) for 16 h at 25 °C and 90% RH and sprouted for 72 h. Kernels were afterward divided into two aliquots and underwent two different drying treatments. The temperatures chosen for drying were 50 °C, commonly used in industrial sprouting, and 40 °C, to simulate sun-drying in Africa, where sprouting is traditionally performed at home. Therefore, one aliquot was dried for 6 h at 50 °C (SSD50), another for 12 h at 40 °C (SSD40) to obtain the same final moisture content for both processes.

Unsprouted (US) and sprouted sorghum were milled using a lab-scale mill (Labormill, BONA, Italy) to produce refined flour, middlings, and bran. Sprouted seed rootlets were recovered before milling and added to the bran. After bran micronization (500µm), the fractions obtained were reconstituted to wholegrain flour, with the following particle mass distribution: ≈23% particle size >300 µm; ≈30% particle size between 300 and 200 µm, ≈27% between 200 and 100 µm and ≈20% particle size <100 µm. The flour samples were stored at 4 °C until analysis.

2.2 Proximate composition

Protein, lipid, ash, and moisture content were assessed in triplicate by AACC standard methods (46-12.01, 30-25.01, 08-01.01, 44-15.02, respectively; AACC 2001), while carbohydrates were determined by difference and the results expressed as % (g per 100 g) dry basis (d.b.).

The fibre content [Total (TDF), insoluble (IDF) and soluble (SDF)] was determined in duplicate using an enzymatic-gravimetric method according to AOAC Official Methods of Analysis 985.29, 991.42, 993.19; AOAC 2003), respectively.

2.3 Protein characterization and functionality

2.4 Protein extraction and fractionation

Sorghum flours were defatted by stirring in 5 vols (w/v) of petroleum ether for 1 h at room temperature. After drying, the flour was reground and petroleum ether extraction was repeated for 30 min. Defatted flour (200 g) was extracted following the procedure described by Hamaker, Mohamed, Habben, Huang, & Larkins (1995) and modified by Park & Bean (2003). Briefly, the flour was extracted for 1 h on a shaker at R/T with 0.0125 M sodium borate buffer, containing 2% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol (β-ME) (pH 10.0) at a solvent-to-sample ratio of 20:1. The suspension was centrifuged twice at 4637 g (6000 rpm) for 25 min (Eppendorf 5810 R, Hamburg, Germany) and the supernatants were pooled. Non-kafirin proteins were precipitated from the total protein extract with 70% ethanol; the mixture was allowed to stand for 2 h with occasional stirring, then centrifuged at R/T for 40 min at 15585 g (11000 rpm). The supernatant contained the prolamin (kafirin) and non-protein nitrogen fractions was collected; the pellet containing non-kafirin proteins was then solubilized in 2 ml Milli-Q water. Protein extracts were quantified using the Bradford method (Bradford, 1976) and bovine serum albumin (BSA, Sigma-Aldrich) as standard protein. Absorbance values from the samples analysed were interpolated in a standard curve equation to obtain protein concentration. Twelve determinations were acquired for both kafirin and non-kafirin extracts.

2.4.1 SDS-PAGE

Aliquots of the protein extracts were concentrated using VIVASPIN 500 (Sartorius Biotech, Goettingen, Germany). 6 µg of kafirins and 20 µg of non-kafirins were analysed by SDS-PAGE on 13% polyacrylamide gel in the presence of 0.13% 2-ME using a vertical electrophoresis system (Mini-PROTEAN Tetra System, Bio-Rad, Hercules, CA, USA). A broad range protein molecular weight standard (10-250 kDa) was used (BioRad, Richmond, CA, USA). Gels were stained with 0.25% Coomassie Brilliant Blue R and the images digitized with a ChemiDoc MP Gel Imaging System scanner (Bio-Rad, USA).

2.5 Amino acid analysis

Sorghum flour (500 mg) underwent acid hydrolysis and derivatization with AccQ Tag (Waters, Milford, MA, USA) as described in Anzani et al. (2018). All samples and standard solutions were analysed using a

UPLC/ESI-MS system as described by Buhler et al. (2019). The ratio of essential amino acid (EA) to total amino acid (TA) was calculated (EA/TA, %).

2.6 Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

WHC and OHC were measured in triplicate according to Marchini et al. (2020). Briefly, 100 mg of flour were mixed with 1 ml of distilled water (WHC) or sunflower oil (OHC), shaken with a vortex for 30 sec, and then allowed to rest at R/T for 30 min. Mixtures were centrifuged at 2061 g (4,000 rpm) for 20 min and the supernatant decanted. WHC and OHC were calculated as the ratio between the grams of water or oil per gram of solid.

2.7 Starch characterization and functionality

2.7.1 Total starch (TS), Resistant Starch (RS), Digestible Starch (DS), Amylose content

Total starch (TS), resistant starch (RS) and digestible starch (DS) were determined using a Megazyme Resistant Starch Assay Kit (K-RAPRS, Megazyme International Ireland Ltd., Wicklow, Ireland) following AACC standard method no. 32-40.01.

Amylose content was quantified in triplicate using the Megazyme amylose/amylopectin assay procedure (K-AMYL 06/18 commercial kit, Megazyme International Ireland Ltd., Wicklow, Ireland) following the manufacturer's protocol. The amylose/amylopectin ratio was then calculated for each sample.

2.7.2 α-amylase activity, pasting and thermal properties and swelling power (S_p)

α-amylase activity was determined according to AACC standard method no. 22-02.01, using the Megazyme Amylase Assay Procedure (K-CERA, Megazyme International Ireland Ltd., Wicklow, Ireland).

Pasting properties of flours were measured in triplicate using a Micro-Visco-Amylograph device (MVAG, Brabender GmbH & Co. KG, Duisburg, Germany) as described by Marti, Cardone, Nicolodi, Quaglia, & Pagani (2017), using a 1 mM aqueous $AgNO_3$ solution instead of distilled water to inhibit α-amylase activation during analysis. Briefly, flour (12 g) was dispersed in 100 mL of a 1 mM aqueous $AgNO_3$ solution and stirred at 250 rpm. Pasting properties were determined applying the following tem-perature profile: heating from 30 °C to 95 °C at a rate of 3 °C/min, holding at 95 °C for 20 min, cooling from 95 °C to 30 °C at a cooling rate of 3 °C/min, and holding at 30 °C for 1 min.

The pasting temperature (temperature at which gelatinization begins, °C), peak viscosity (maximum viscosity value of the slurry during gelatinization, BU), peak temperature (temperature at which peak viscosity occurs, °C), final viscosity (viscosity of the slurry at the end of the test, BU), breakdown (difference between peak viscosity and minimum viscosity during the holding period, BU) and setback (difference between peak and final viscosities, BU) were calculated from the pasting curve.

Thermal properties of flours were measured in triplicate using a differential scanning calorimeter (DSC Q100 TA Instruments, USA), calibrated with indium (melting point: 156.6 °C, melting enthalpy: 28.71 J/g) and mercury (melting point: − 38.83 °C, melting enthalpy: 11.44 J/g), as described by Marchini et al. (2020).

Briefly, flour and distilled water were mixed in a ratio of 1:3 w/v and left to equilibrate overnight at R/T. An aliquot of the water-flour suspension (5-10 mg) was placed in stainless steel pans (Perkin Elmer, USA) hermetically sealed, quench cooled to 30 °C and then heated to 120 °C at 5 °C/min, using an empty pan as reference. The enthalpy (ΔH, J/g), onset (T_{on}, °C), peak (T_p), and offset (T_{off}, °C) temperatures of the observed transitions were obtained from heat flow curves using Universal Analysis Software, Version 4.5A (TA Instruments, USA).

S_P was measured as described previously by Marchini et al. (2020). Briefly, flour suspensions (2% w/v) were heated in a water bath at 60, 70, 80 or 90 °C for 1h and cooled at 30 °C for 30 min. The samples were centrifuged at 8,243 g (8,000 rpm) for 20 min and the precipitate weighed. S_P was calculated as the ratio between sediment and dry sample weights. Determinations were in triplicate.

2.8 Microstructure

The size and distribution of sorghum flour sample cells were examined using optical microscopy (DM 4000B, LEICA, Wetzlar, Germany). Flour particles were stained using toluidine blue (0.1%); 6 slides per flour were analysed. Images of cells (6) and cell agglomerates (18) were observed at 20× and 1.25× respectively and acquired by a camera (Leica DMC2900, Germany). Cell aggregate areas were obtained using imaging analysis software (Leica, IM50 Version 4.1, Germany). Agglomerates were arbitrarily divided into four dimensional classes, as follows: Class I: 1-50,000 μm^2; Class II: 50,001-100,000 μm^2; Class III: 100,001-150,000 μm^2; Class IV: >150,000 μm^2, and the average number of representative agglomerates for each class calculated. The number of cells per agglomerate was calculated by dividing the average aggregate area by the mean cell area. Ultrastructural analysis of flours was performed with an Environmental Scanning Electron Microscope Quanta™ 250FEG ESEM (FEI, Hillsboro, USA). The samples, after fixing to a stub with carbon double-sided tape, were directly analysed in low vacuum mode (pressure chamber at 70 Pa) with a beam accelerating voltage of 5 kV. Magnification ranges of ESEM micrographs (≥10 micrographs acquired) were 3,000-6,000×.

2.9 Statistical analysis

To determine significant differences between samples, data were analysed by *one-way analysis of variance* (ANOVA) followed by Duncan's post-hoc test at 0.05 significance level. Analyses used SPSS Statistical Software (Version 25.0, IBM SPSS Inc., USA).

3. Results and discussion

Table 1 Proximate composition, physico-chemical and pasting properties of unsprouted and sprouted sorghum flours. Compositional data are expressed as g/100 g dry basis (d.b.). In brackets, SDF referred to g/100 g d.b. TDF; RS and DS referred to g/100 g d.b. TS.

	US	SSD50	SSD40
Protein (g/100 g d.b.)	11.27 ± 0.31b	10.98 ± 0.48b	11.97 ± 0.00a
Fat (g/100 g d.b.)	3.71 ± 0.03a	3.25 ± 0.00b	3.07 ± 0.02c
Moisture (g/100 g w.b.)	11.31 ± 0.15b	12.69 ± 0.04a	12.67 ± 0.03a
Ash (g/100 g d.b.)	1.35 ± 0.02a	1.30 ± 0.00c	1.33 ± 0.00b
Carbohydrates (g/100 g d.b.)	85.25 ± 0.12a	84.48 ± 0.53b	83.63 ± 0.07c
Total Dietary Fibre (TDF, g/100 g d.b.)	7.4 ± 0.67	7.1 ± 0.5	7.4 ± 0.7
Insoluble (IDF, g/100 g d.b.)	7.3 ± 0.8 (98.65)	7.1 ± 0.5 (98.59)	7.1 ± 0.85 (95.95)
Soluble (SDF, g/100 g d.b.)	< LQ	< LQ	< LQ
WHC (g/g)	1.51 ± 0.05b	1.52 ± 0.08b	1.68 ± 0.05a
OHC (g/g)	0.95 ± 0.01b	1.00 ± 0.07ab	1.05 ± 0.00a
Total Starch (TS, g/100 g d.b.)	73.7 ± 0.0a	69.9 ± 0.1b	69.1 ± 0.2c
Digestible (DS, g/100 g d.b.)	66.9 ± 0.0a (90.8)	65.3 ± 0.0b (93.5)	64.8 ± 0.3c (93.7)
Resistant (RS, g/100 g d.b.)	6.8 ± 0.0a (9.2)	4.6 ± 0.1b (6.6)	4.3 ± 0.2c (6.3)
Amylose (%)	29 ± 3b	34 ± 4b	47 ± 0a
Amylose/Amylopectin ratio	0.41	0.51	0.88
α-amylase activity (CU/g)	0.06 ± 0.01b	22.60 ± 0.85a	20.64 ± 0.88a
Pasting properties (MVAG Test)			
Pasting temperature (°C)	78.7 ± 0.3b	80.9 ± 1.1a	76.5 ± 0.1c
Peak viscosity (BU)	269.5 ± 3.5a	187.0 ± 6.0b	92.5 ± 5.5c
Peak temperature (°C)	93.7 ± 1.3a	92.2 ± 0.8ab	89.9 ± 1.9b
Final viscosity (BU)	643.5 ± 4.5a	335.5 ± 1.5b	105.0 ± 11.0c
Breakdown (BU)	80.0 ± 2.0a	74.5 ± 6.5b	47.5 ± 6.5c
Setback (BU)	482.5 ± 5.5a	247.50± 15.5b	59.0 ± 17.0c
S_P (g/g)			
60 °C	5.33 ± 0.33aB	5.39 ± 0.24aC	4.08 ± 0.43bC
70 °C	5.32 ± 0.32B	5.35 ± 0.23C	5.01 ± 0.33B
80 °C	8.36 ± 0.22aA	6.86 ± 0.24bB	5.57 ± 0.07cA
90 °C	8.23 ± 0.15aA	7.63 ± 0.13bA	6.08 ± 0.14cA

US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; WHC, water holding capacity; OHC, oil holding capacity; S_p, swelling power; CU, ceralpha units. Values are expressed as mean ± SD ($n \geq 3$; n=2 for α-amylase activity). Different lowercase letters indicate significant differences (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$) between different samples at the same temperature. For S_p, different capital letters indicate a significant difference (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$) between temperatures for the same sample.

3.1 Proximate composition

Proximate composition for US agreed with the literature (Anyango, & Taylor, 2019) differing significantly (*p* ≤0.05) from that of the sprouted samples (Table 1). Moisture content was comparable in sprouted samples, and higher than US. Additionally, the data revealed a slight increase in protein content for SSD40 only (+6.2%), while SSD50 did not differ significantly from US. Moreover, the sprouting significantly (*p* ≤0.05) decreased the fat (-12.4% and -17.3% for SSD50 and SSD40, respectively), ash (-3.7% and -1.5% for SSD50 and SSD40, respectively), and carbohydrate (-0.9% and -1.9% for SSD50 and SSD40, respectively) contents compared to US, in agreement with Lemmens et al. (2019) who reviewed the effect of sprouting on cereals. Changes in proximate composition were always greater in SSD40 than in SSD50. The ~6% increase in protein showed by SSD40 compared to US may be due to release of a much greater amount of free amino acids through protein synthesis in the embryo (Afify et al., 2012). Anyway, a relative difference in protein content between sprouted and unsprouted cereals of less than 10% can be considered negligible (Lemmens et al., 2019). The significant decrease (*p* ≤0.05) in lipid content has to be related to hydrolysis of fat components into fatty acids and glycerol caused by the lipase activity activated during sprouting (Singh et al., 2015), which leads them into the metabolic gluconeogenesis pathway.

Conceivably, the higher fat content found in SSD50 flour may be caused by a higher lipase activity inactivation consequent to the higher drying temperature compared to SSD40, therefore closer to the lipase inactivation range (60-80 °C, Ekstrand, Gangby, & Åkesson, 1992).

A decrease in macronutrients on sprouting has also been reported by Afify et al. (2012). However, the different time/temperature combination of the drying treatment which the sprouted grain underwent, differently affected the enzymatic and metabolic processes activated during sprouting, causing the observed slight but significant differences in the proximate composition of the flours.

Sprouting processes did not affect either TDF or IDF (which comprises more than 95% of TDF) content of sorghum to any great extent (Table 1). The results suggest that the processes used did not significantly modify the cell walls, as verified by optical microscopy images (see paragraph 3.4); the IDF found in the outer layer of the grain is conceivably little prone to degradation during sprouting and heating. Similar dietary fibre composition as a function of sorghum sprouting can be found in the literature (Anyango, & Taylor, 2019).

3.2 Protein characterization and functionality

3.2.1 Protein extraction and fractionation

The protein extraction procedure used in this study was chosen on the basis of the authors’ suggestion that a reasonable – and simpler – classification of sorghum proteins would be to divide them into kafirin and non-kafirin groups. This classification reflects the homogeneous nature of the kafirin storage prolamins, as opposed to the heterogeneous group of non-kafirins (namely, albumins, globulins and glutelins), involved in cellular functions (De Mesa-Stonestreet et al., 2010). This procedure was effective for the extraction and fractionation of both kafirins and non-kafirins, as confirmed by colorimetric determinations performed on protein extracts. Specifically, protein concentrations (given by the Bradford method) in the kafirin extracts were 0.29±0.04

µg/µL, 0.33±0.02 µg/µL and 0.48 ±0.06 µg/µL for US, SSD50 and SSD40, respectively, while protein concentration in non-kafirin extracts was 4.66±0.62 µg/µL, 7.99±0.75 µg/µL and 6.67±0.38 µg/µL for US, SSD50 and SSD40, respectively.

3.2.2 SDS-PAGE

The kafirins and non-kafirins extracted from sorghum flours were evaluated using SDS-PAGE, as shown in Fig. 1.

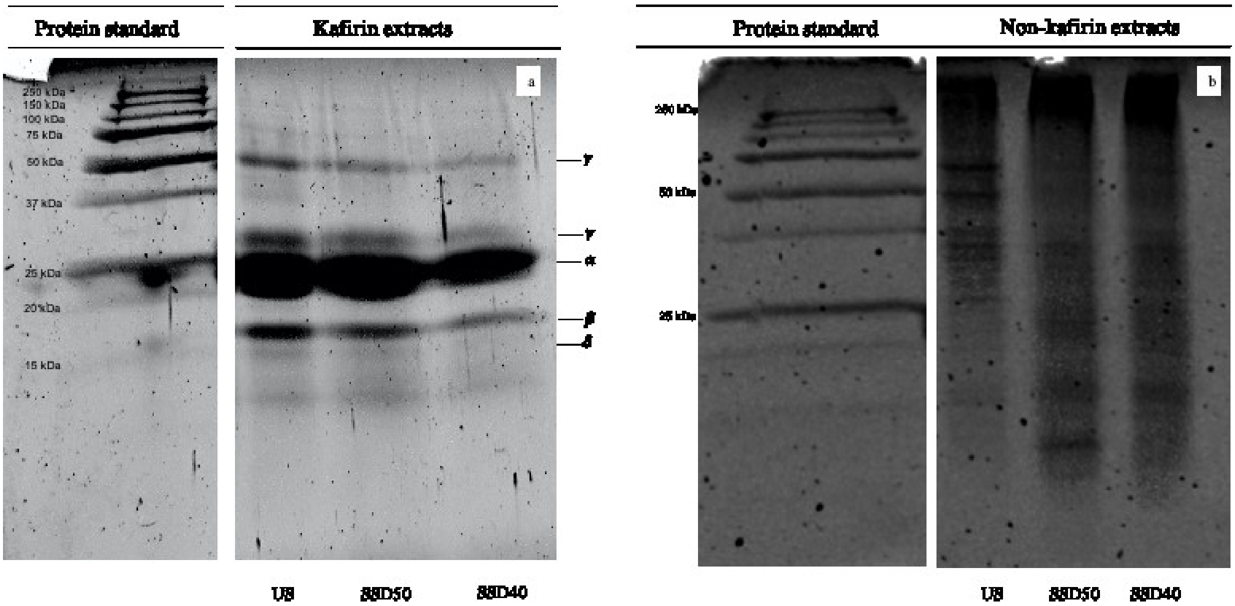

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of kafirin (a) and non-kafirin (b) fractions of sorghum flours. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

The electrophoretic system showed a typical reduced kafirin electrophoretic profile (Fig. 1a), characterized by the absence of any lanes generated by high molecular weight (HMW) aggregates and >50 kDa oligomers. Indeed, reducing conditions caused an S-S bond cleavage which disrupts the oligomers, leading to the appearance of low molecular weight constituents (El Nour, Peruffo, & Curioni, 1998). Accordingly, Fig. 1a illustrates three main fractions with a molecular weight (M_r) of 16-18 kDa, 23-25 kDa and 27-28 kDa, corresponding to β-, α-, and γ-kafirin, respectively (El Nour et al., 1998; De Mesa-Stonestreet et al., 2010). The lane generated by α-kafirin predominated and was created by the overlapping of two bands generated by two subunits (α_1- and α_2-kafirin) with slightly different mobility (El Nour et al., 1998). Furthermore, the visible minor band of M_r at about 49 kDa (Fig. 1a) had previously been attributed to an unreduced oligomer of γ-kafirin (Belton, Delgadillo, Halford, & Shewry, 2006), while from among the fainter low molecular weight lanes identified at $M_r \leq 15$ kDa, Belton et al. (2006) detected the 14 kDa δ-kafirin component.

As expected, a decrease in prolamin electrophoretic lane intensities was observed upon sprouting (Fig. 1a), especially in those generated by β-, and γ-kafirin. This evidence is consistent with the literature and has been related to prolamin hydrolysis (Correia et al., 2008; Georget, Elmoneim, & Peter, 2012). Given that kafirin protein bodies consist in an outer “shell” composed mainly of crosslinked β- and γ-kafirins and a core mainly containing α-kafirin (De Mesa-Stonestreet et al., 2010), β- and γ-kafirins are the first proteins to degrade during sorghum sprouting. Since this degradation begins from the surface, the β- and γ-kafirins breakdown may be explained by their peripheral location (Georget et al., 2012). The observed decrease in prolamin electrophoretic lane intensities was more pronounced in the SSD40 kafirin extract (Fig. 1a), which may be due to a higher prolamin hydrolysis than occurs in SSD50 during sprouting.

The greater differences in SSD40 may have been caused by the drying conditions used: 40 °C and 50 °C proved to be close to the optimum temperature range for cereal protease activity (Phiarais et al., 2005), while the SSD40 prolonged drying time may have caused not only a failing enzymatic inactivation effect, but also prolonged enzyme activity over time.

The electrophoretic analysis of non-kafirin protein fraction (Fig. 1b) showed a profile characterized by a broader number of bands distributed throughout the electrophoretic gel, attributable to HMW aggregates, oligomers and monomers of albumins, globulins and glutelins, which constitute around 10% of wholegrain sorghum flour proteins (Hamaker et al., 1995) in agreement with data in the literature (Hamaker et al., 1995; Mokhawa, Kerapeletswe-Kruger, Ezeogu, 2013).

Compared to US, small changes were observed in the SDS-PAGE analysis of non-kafirin extracts on sprouting (Fig. 1b). SSD50 and SSD40 non-kafirin extracts exhibited a slight decrease in the intensity of lanes characteristic of HMW aggregates in favour of an increase in the intensity of oligomer and monomer bands (≤50 kDa), confirming the effect of proteolytic activity (Mokhawa et al., 2013).

3.3 Amino acid analysis

The amino acid profile of unsprouted and sprouted sorghum (Table 2) was found overall comparable with those reported in the literature (Mokrane, Amoura, Belhaneche-Bensemra, Courtin, Delcour, & Nadjemi, 2010).

Table 2 Amino acid composition of sorghum flours expressed as g/100 g flour. In brackets, the % index of change compared to S value.

Amino acid (g/100 g flour)	US	SSD50	SSD40
Essential amino acids (EA)			
Histidine	0.193 ± 0.011	0.210 ± 0.005	0.204 ± 0.005
Isoleucine	0.372 ± 0.005 b	0.386 ± 0.007 ab (+3.76)	0.391 ± 0.010 a (+5.11)
Leucine	1.256 ± 0.021	1.232 ± 0.027	1.276 ± 0.029
Lysine	0.147 ± 0.002 c	0.153 ± 0 b (+4.08)	0.160 ± 0.001 a (+8.84)
Methionine	0.169 ± 0.011 b	0.174 ± 0.003 b (+2.96)	0.189 ± 0 a (+11.83)
Phenylalanine	0.550 ± 0.007	0.544 ± 0.005	0.561 ± 0.036
Threonine	0.315 ± 0.008	0.327 ± 0.002	0.328 ± 0.007
Valine	0.500 ± 0.011	0.476 ± 0.032	0.518 ± 0.010
Cysteine	0.188 ± 0.002 b	0.190 ± 0.005 b (+1.06)	0.198 ± 0.002 a (+5.32)
Tyrosine	0.243 ± 0.001	0.255 ± 0.002	0.260 ± 0.022
E/T (%)	43.5	43.2	42.7
Non-essential amino acids (NEA)			
Alanine	0.801 ± 0.020 ab	0.785 ± 0.010 b (-2.00)	0.830 ± 0.018 a (+3.62)
Arginine	0.283 ± 0.014	0.289 ± 0.011	0.282 ± 0.007
Aspartic acid	0.586 ± 0.023 c	0.656 ± 0.008 b (+ 11.95)	0.707 ± 0.037 a (+20.65)
Glutamic acid	1.898 ± 0.103	1.861 ± 0.015	1.962 ± 0.066
Glycine	0.317 ± 0.004	0.303 ± 0.002	0.323 ± 0.014
Hydroxyproline	0.004 ± 0	0.005 ± 0.001	0.005 ± 0
Proline	0.782 ± 0 c	0.852 ± 0.023 b (+8.95)	0.908 ± 0.021 a (+16.11)
Serine	0.437 ± 0.002 b	0.447 ± 0.011 ab (+2.29)	0.464 ± 0.012 a (+6.18)
Total	9.040 ± 0.190 b	9.142 ± 0.142 b (+1.13)	9.564 ± 0.056 a (+5.80)

Values are expressed as mean ± SD (n=3). Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

With a value higher than 0.5 g/100 g flour, the major amino acids are leucine, phenylalanine, valine (essential amino acids, EA), alanine, proline and aspartic and glutamic acids (non-essential amino acids, NEA). Moreover, a favourable amino acid balance determined by high levels of EA (EA/TA % ~43%) was found, confirming sorghum as a high-value product from a nutritional point of view (Mokrane et al., 2010).

As expected, the percentage of hydrophobic AA (isoleucine, leucine, methionine, phenylalanine, valine, alanine, hydroxyproline and proline) accounted for ~49% of the total, thus confirming the hydrophobic characteristics of sorghum proteins, especially kafirins (De Mesa-Stonestreet et al., 2010). The second most abundant AA were the acidic ones (aspartic and glutamic acid) which represented ~28% of the total, followed by the uncharged polar AA (glycine, serine, threonine, tyrosine, and cysteine) accounting for ~16.5 % and the basic amino acids (lysine, arginine, histidine) representing 7%. An increase in several AA was observed after sprouting (Table 2) with values in agreement with the literature (Afify et al., 2012) which has also reported an improved amino acid profile on sprouting. During the metabolic processes activated during sprouting for the developing embryo, AA may be produced in excess of requirements and tend to accumulate in the free amino acid pool (Afify et al., 2012). Amino acids, especially those which increase during sprouting, are involved in such fundamental physiological plant activities and metabolic pathways, as protein and secondary metabolites synthesis, which in turn play a vital role in plant growth, regulation of plant metabolism, resistance to abiotic, water and osmotic stresses, increases in germinability as precursors of growth factors, development of new tissues, and hormonal-like activities (Gan, Lui, Wu, Chan, Dai, Sui, & Corke, 2017).

In any case, it was interesting to observe that changes to AA profiles after sprouting were drying-treatment-dependent. The total AA content significantly increased only in SSD40 (+5.8%) compared to US, while SSD50 showed no significant differences compared to US. Among the EA, the isoleucine content of SSD40 showed a greater increase (5.1%) than SSD50 (3.8%), while the change in lysine was similar to that of isoleucine except that the amplitude was larger (8.8% and 4.1% for SSD40 and SSD50, respectively). The increase in methionine and cysteine content measured in SSD40 was around 6 times higher than in SSD50 (11.8% vs 3% and 5.3% vs 1.1%, respectively). As for the EA, SSD40 showed a twofold increase in aspartic acid compared to SSD50 (20.7% *vs*. 12%), proline (16.1% *vs*. 9%) and serine (6.2% *vs*. 2.3%). In SSD40, also the alanine content increased (3.6%) compared to US, while SSD50 showed a decrease (2%). Therefore, the drying conditions may also have influenced the amino acid composition of the flour. It has been hypothesized that the treatment carried out at 50 °C for 6 h (SSD50) may also have caused higher AA degradation than that carried out at a lower temperature (40 °C for 12 h, SSD40).

3.4 Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

The US sample showed a WHC (Table 1) within the range previously identified in the literature (Mahajan, & Gupta, 2015). Sprouting significantly increased WHC ($p \leq 0.05$) only in the case of SSD40.

Likewise, sprouting processes significantly increased the OHC of sorghum flours ($p \leq 0.05$), with SSD40 showing the higher values. Data were within the range identified by Elkhalifa, & Bernhardt (2010) for sprouted sorghum.

The impact of sprouting on WHC and OHC have already been well documented (Elkhalifa, & Bernhardt, 2010; Singh, Rehal, Kaur, & Jyot, 2015; Mahajan, & Gupta, 2015) and also related to changes in protein functionality due to the proteolytic enzymes activated during sprouting, which give rise to an improved capacity to retain water and fat globules (Singh et al., 2015; Lemmens et al., 2019; Elkhalifa, & Bernhardt, 2010). Specifically, changes in hydration properties may be due to increased polar groups in proteins and polysaccharides upon sprouting. The exposure of part of the water binding site on the side chain groups of proteins seemingly leads to an increase in sites which interact with water and a corresponding increase in WHC in sprouted samples (Singh et al., 2015).

Similarly, a higher OHC appears to be due to the dissociation and partial unfolding of polypeptides which exposes the hydrophobic amino acids sites while favouring hydrophobic association of peptide chains with lipid droplets (Singh et al., 2015). Therefore, the decreased fat content in the sprouted samples may have been due to the ability of proteins to absorb more oil. Consequently, the higher WHC and OHC recorded for SSD40 may be related to a more intensive protein hydrolysis in this sample, as discussed above (Paragraph 3.1.2).

3.5 Starch characterization and functionality

3.5.1 Total Starch (TS), resistant starch (RS), digestible starch (DS), amylose content

The effect of sprouting on sorghum starch structure was assessed by RS, DS and amylose content determinations. The starch content of native sorghum flour was 73.7 g/100 g d.b. (Table 1), of which 9.24% was RS, in line with the literature (Kulamarva et al., 2009; De Carvalho Teixeira et al., 2016). Furthermore, in native sorghum flour, DS was found to be 90.8% of TS. As expected, a decrease in TS (~5%) occurred in both SSD50 and SSD40 (Table 1), due to the starch-degrading enzymes activated and *de novo* synthesized during sprouting. An increase in DS (Lemmens et al., 2019) with a decrease in RS upon sprouting was found previously in cereals (Gamel, Linssen, Mesallem, Damir, & Shekib, 2005). Notably, the percentage of RS out of TS content decreased to 6.6% in SSD50 and 6.3% in SSD40, showing that the latter had slightly higher starch digestibility. Indeed, the percentage of DS with respect to TS increased slightly more in SSD40 than in SSD50 (93.7 and 93.5%, respectively).

The increase in starch digestibility found in the sprouted samples may be due to protein hydrolysis and breakage of the disulphide bond cross-linking involving kafirins in the protein matrix, thereby rendering the starch granules more susceptible to enzymatic action (Kulamarva et al., 2009). Furthermore, in accordance with Lemmens et al. (2019), starch digestibility may have increased upon sprouting because of the higher content of enzymatically damaged starch granules, thin cell walls, and more readily available sugars. An increase in starch digestibility makes sprouted sorghum flour suitable to produce food for infants, the elderly, or undernourished people who require a readily available source of energy (Lemmens et al., 2019), or to fortify staple foods (e.g., flatbread) for developing countries to potentially increase their nutritional value. Overall, as shown by the higher DS found in SSD40 than SSD50, also drying treatment after sorghum sprouting can further improve the starch digestibility of the product.

Amylose content (%) and amylose/amylopectin ratios of the flours are reported in Table 1. Unsprouted sorghum flour data agreed with the literature (Zhu, 2014). Sprouting increased amylose content (therefore, the amylose/amylopectin ratio) especially for SSD40 treatment. Indeed, increased amylose content was observed in both sprouted samples (+17% and +62% for SSD50 and SSD40, respectively), even if US and SSD50 did not differ statistically. The increase in amylose content and amylose/amylopectin ratio may be due to the preferential hydrolysis of amylopectin chains and cleavage of its long chain branches by amylases *de novo* synthesized and activated during sprouting (Muralikrishnaa, & Nirmala, 2005). The higher amylose/amylopectin ratio found for SSD40 compared to SSD50 documented the higher, more extensive hydrolytic activity which SSD40 underwent, conceivably related to increased starch accessibility thanks to the higher protein matrix degradation observed in this sample, as discussed previously.

3.5.2 α-amylase activity, pasting and thermal properties, swelling power (S_p)

As expected, the sprouted samples featured much more enzymatic activity than US, with no significant differences between the two sprouted samples (Table 1). Data confirmed the *de novo* synthesis and accumulation of α-amylase in scutellum and aleurone cells during sprouting, leading to partial hydrolysis of starch into sugars which are an energy source for the developing embryos (Lemmens et al., 2019). The comparable values between the two samples suggests that drying was able to promote α-amylases activity over the treatment time in the same way, possibly due to the optimal temperature ranges for enzyme activity (Muralikrishnaa, & Nirmala, 2005) in both drying treatments. Despite this, as shown previously, the starch fraction was differently affected by drying as a result of a different protein matrix degradation which conceivably affected starch accessibility.

The presence of endogenous α-amylase strongly impacted the samples' pasting profile (Fig. 2), since the temperature profile of the analysis caused enzyme activation and thus starch hydrolysis (Mariotti, Zardi, Lucisano, & Pagani, 2005). Accordingly, amylases were inhibited with a 1 mM aqueous $AgNO_3$ solution to understand any pasting profile changes due to experimental variables (Mariotti et al., 2005).

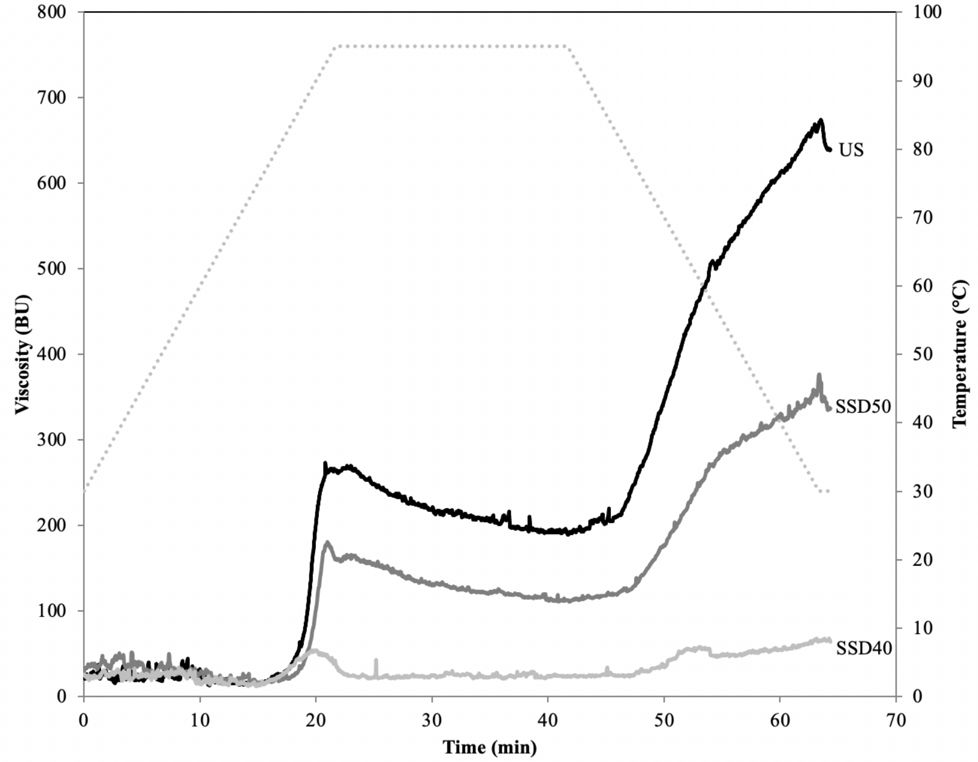

Fig. 2. Pasting properties of sorghum flour samples measured by means of a Micro-Visco Amylograph. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

The pasting temperature recorded for the US sample was 78.8 ±0.3 °C; SSD50 showed a significantly ($p \leq 0.05$) higher value, indicating the prerequisite of higher temperatures to reach full granule swelling, while a decrease in SSD40 was found (Table 1). Since it is the starch structure which governs the initial gelatinization point and the range the gelatinization occurs over, pasting temperature shifts were apparently caused by modification of starch granule structure, as reported elsewhere (Marengo et al., 2015).

As expected, a peak viscosity decrease occurred upon sprouting, SSD40 showing the greater decrease (Table 1 and Fig. 2). This is due to several factors, including starch degradation, debranching to simpler units, and changes in proteins and fatty acids (Zhang & Hamaker, 2005; Sun et al., 2014). The marked viscosity loss in the samples was accompanied by a peak temperature decrease, supporting the assumption that extensive starch breakdown caused by endogenous enzymes had occurred during sprouting, especially during SSD40 treatment. Correspondingly, sprouting also provoked a final viscosity decrease, with SSD40 showing the lowest value. Additionally, both samples showed a lower breakdown value than US, suggesting a starch heat-stability increase (Marengo et al., 2015).

The setback value – reflecting the retrogradation tendency of amylose in starch paste – decreased in both sprouted samples, suggesting a decrease in starch retrogradation ability compared to US. During sprouting, the outermost branches of amylopectin are hydrolysed by α-amylase and are thus no longer able to form large amylopectin crystals. These small crystallites cannot form a three-dimensional network capable of promoting a major increase in viscosity during cooling (Marti et al., 2017). This trend could prove of interest to the food industry and bakery sector given that low setback values indicate a low rate of starch retrogradation and syneresis.

Overall, sprouting resulted in a significant decrease ($p \leq 0.05$) in viscosity during the heating and cooling phases as a consequence of degradative processes on starch phase activated by the sprouting. The different pasting behaviour between SSD50 and SSD40 may be attributed to the different magnitude of enzyme activity inhibition that occurred during drying. SSD50 still had the ability to form a gel below 95 °C, which is interesting with a view to formulating products using sprouted sorghum with improved nutritional and technological properties. Indeed, SSD40 did not show a typical pasting profile despite the addition of $AgNo_3$ as inhibitor; in particular, there was no real viscosity peak and the curve remained flat throughout the analysis as previously observed on other cereals (Marengo et al., 2015). The lower drying temperature condition could therefore find application in food formulation where extensive gelatinization of starch is not required (e.g., flatbread) with the advantage of providing improved starch digestibility.

The swelling power (S_p) of the flours was determined at four different heating temperatures to better reflect the structural changes to starch functionality as a result of the sprouting treatments (Table 1). As expected, S_p showed a continuous increase as the temperature rose in all the samples in agreement with previous studies (Sun et al., 2014). Moreover, the data revealed that S_p significantly decreased as a consequence of the sprouting, as previously reported on other cereals (Singh et al., 2017). Among the sprouted samples, SSD40 showed the lowest S_p values at all temperatures. At 60 °C, US and SSD50 presented a comparable S_p value, while SSD40 had a significantly lower S_p ($p \leq 0.05$). Nevertheless, at 70 °C, the S_p of SSD40 increased to become comparable with that of the other two flours. A more substantial increase in S_p in all samples when the temperature began to reach ~80 °C was found, as expected (Li, & Yeh, 2001), while a further increase in temperature (90 °C) did not significantly modify the flours' S_p.

It is widely accepted that amylopectin is the primary component responsible for starch S_p and is also the component mainly responsible for the formation of starch crystalline structure (Li, & Yeh, 2001). The reduction in S_p may be due to changes in both starch content (Table 1) and structure (i.e., amylose/amylopectin ratio) due to enzymatic activities. In addition, degradation by α-amylase causes accumulation of dextrin, oligosaccharides and fermentable sugars which have no S_p, thereby interfering with the starch formation of more compact gels (Singh et al., 2017). Overall, the difference in S_p found between SSD40 and SSD50 may be attributed to the different degree of starch and amylopectin degradation during sprouting and drying.

DSC representative thermograms and thermal properties of the flours are shown in Fig. 3 and Table S1, respectively.

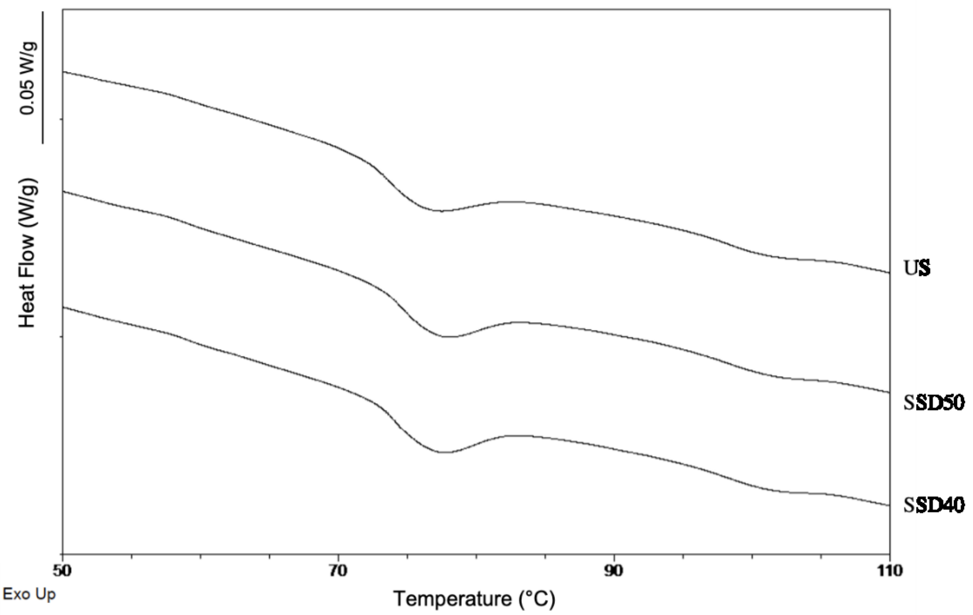

Fig. 3. Representative DSC thermograms of US, SSD50 and SSD40 flours in the range 50-110 °C. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

Two endothermic events were evident for all flours (Fig. 3), with the main thermal transition found between ~58 °C and ~91 °C, corresponding to starch gelatinization. Thermograms of sprouted samples also showed a minor endothermal event between ~58 and ~65 °C, likely related to a small fraction of starch gelatinizing at a lower temperature.

Average gelatinization temperatures agreed with those in the literature, while the gelatinization enthalpy was lower (Sun et al., 2014; Zhu, 2014) possibly due to genetic and/or environmental factors (Zhu, 2014).

As seen in Table S1, a significative ($p \leq 0.05$) increase in T_{on}, a decrease in T_{off} and a decrease in enthalpy occurred upon sprouting with no significant differences between the two sprouted samples.

The third endothermic event was found in the temperature range ~94-108 °C (Fig. 3) similar to that reported in the literature and related to amylose-lipid complexes melting (Zhu, 2014). The slightly higher amylose-lipid complexes melting enthalpy recorded by SSD40 may be related to its higher amylose content (Table 1). Overall, in all the endothermic peaks identified, the thermal parameters showed no significant variations as a result of the drying treatment. These results suggested that the experimental variables did not affect the flours' thermal properties at a mesoscopic level in conditions of excess water. Conversely, as shown previously, the same experimental variables significantly affected pasting properties at the macroscopic level when measured with an empirical approach.

3.6 Microstructure

Optical microscopy was used to investigate the effect of sprouting and drying on cell aggregates and cell wall integrity (Fig. S2). Indeed, during cereal sprouting and subsequent drying, an enzymatic and/or thermal degradation of the cell wall's non-starchy polysaccharide components may occur (Palmer, 1991; Wu, Bulgakov, & Jinn, 2018).

For all flours, the cells were sorted into different-sized aggregates (Figs. S1b, S1d, S1f) with heterogeneous dimension distribution (Figs. S1a, S1c, S1e). For all flours, the majority of cell aggregates (~60%) were small in size (1-50,000 μm^2), ~20 % of the agglomerates were in the second dimensional class, ~11 % were in class III, while only 9% of the agglomerates had an area >150,000 μm^2. Fig. S2 (right) shows the average number of cells per agglomerate, calculated by dividing the area of each aggregate by the calculated mean area of the cells (8,598 μm^2). The number of cells per agglomerate belonging to different classes did not differ among the samples, indicating that neither the sprouting nor the drying treatment had caused degradation of the pectin-rich cell wall fractions in any of the cases. The absence of significant differences in the cell morphology in different samples indicates that the impact of the various treatments on the sorghum was negligible.

Fig. 4 shows ESEM images of sorghum flours. US (Figs. 4a and 4b) was mainly constituted of compacted and intact starch granules ranging from approximately 15 to 25 μm in size, polygonal in shape, surrounded by spherical protein bodies of around 1 μm in diameter, and enclosed in a compact protein matrix to which protein bodies were attached (Fig. 4a). The starch and protein dimensions and micrographs were similar to those in previously published works (Correia et al., 2008; Zhu, 2014). The ESEM images of the sprouted flours (Figs. 4c, 4d, 4e, 4f) revealed that sprouting affected the sorghum ultrastructure to a different extent depending on the type of drying treatment used. In both SSD50 and SSD40, the proteolytic activity which occurred during sprouting determined a partial degradation of the proteinaceous coating (Figs. 4d and 4f) leading to a release of starch granules and protein bodies. In addition, the protein bodies seemed more detached from the starch granules if compared with US flour. In SSD50, the starch granules appeared eroded in the sites where the protein bodies were located (Figs. 4c and 4d), while in SSD40 the protein bodies seemed no longer visible (Fig. 4e). Additionally, partial hydrolysis of the starch granules was visible in both sprouted samples but appeared more substantial in SSD40 (Figs. 4d and 4e), confirming the data on protein and starch enzymatic degradation, and suggesting that this sample was conceivably more affected by amylolytic activity.

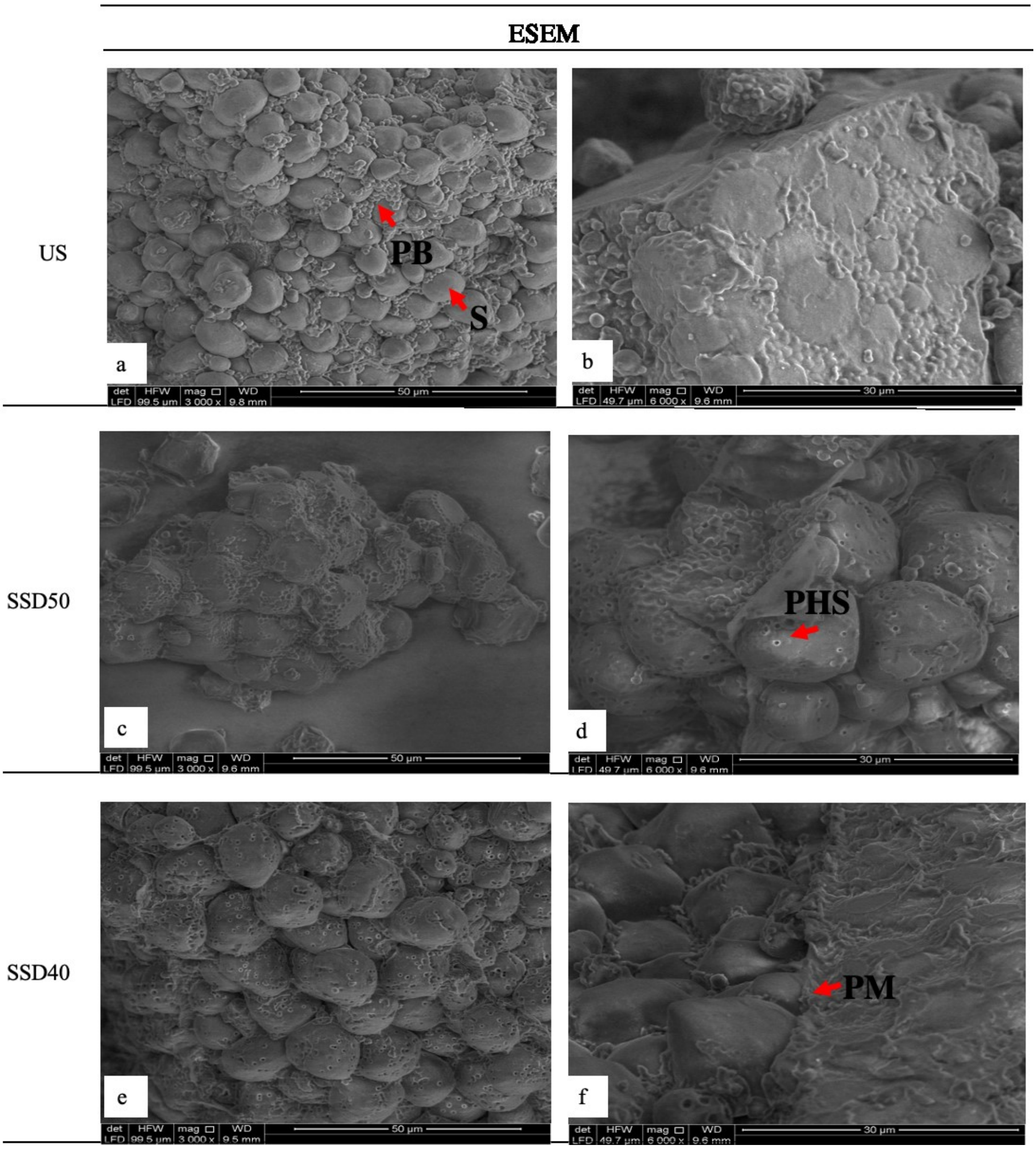

Fig. 4. Morphological observations of sorghum flours acquired with ESEM. Micrographs of US, SSD50 and SSD40 flours were acquired with a magnification of 3000x (a, c, e) and 6000x (b, d, f). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; S, starch granule; PB, protein body; PHS, partially hydrolyzed starch; PM, protein matrix.

4. Conclusion

Two drying treatments were selected and used after sorghum sprouting. The effects on protein and starch features were analysed to determine if the drying treatment could contribute to an improvement in the final product functionality.

Sprouting caused significant changes in the flours' properties, improving their nutritional profile, confirmed by the increase in total amino acid content. Moreover, increased WHC and OHC and worsened starch gelatinization underscore that sprouting also affected protein and starch flour functionality.

Significant differences in the flours' functional and nutritional properties were also found as a function of the drying temperature. The treatment performed at a lower temperature (40 °C) and longer time (12 h) seemed to have favoured extended enzymatic activity over time. Indeed, a higher protein hydrolysis was found in this sample resulting in increased capacity to hold water and fat globules. Additionally, the higher protein matrix deterioration may have caused greater starch exposure to the enzymes leading to higher hydrolysis and increased digestibility. Nevertheless, this worsened starch functionality.

Overall, the drying treatment performed on the sprouts could represent an effective, sustainable method, in addition to the sprouting phase in *sensu stricto*, to improve the nutritional profile of sorghum. Further studies are needed to investigate the effect of the two treatments on the content and accessibility of sorghum micronutrients and bioactive compounds. This insight could be useful to provide a broader overview on the effective potential of drying treatment performed at 40 °C to improve a product's nutritional properties. Moreover, the study of additional post-sprouting drying conditions may be of interest to provide further processing indications useful to modulate nutritional and technological functionalities of final products.

From a technological point of view, these sprouted flours could be used where high starch performance is not required, e.g., unleavened bread. Future research is also needed to further investigate the molecular and rheological properties of derived sprouted sorghum flour-based dough, in order to optimize and use sprouting – an inexpensive cost-effective technology – to market finished products with an improved nutritional profile.

Overall, the outcomes of the sprouting process performed with a drying treatment at a temperature of 40 °C may be of great relevance for those countries where sorghum is a staple food, sprouting is performed at household level and the sprouts are dried in the sun.

Acknowledgments

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Supplementary Material

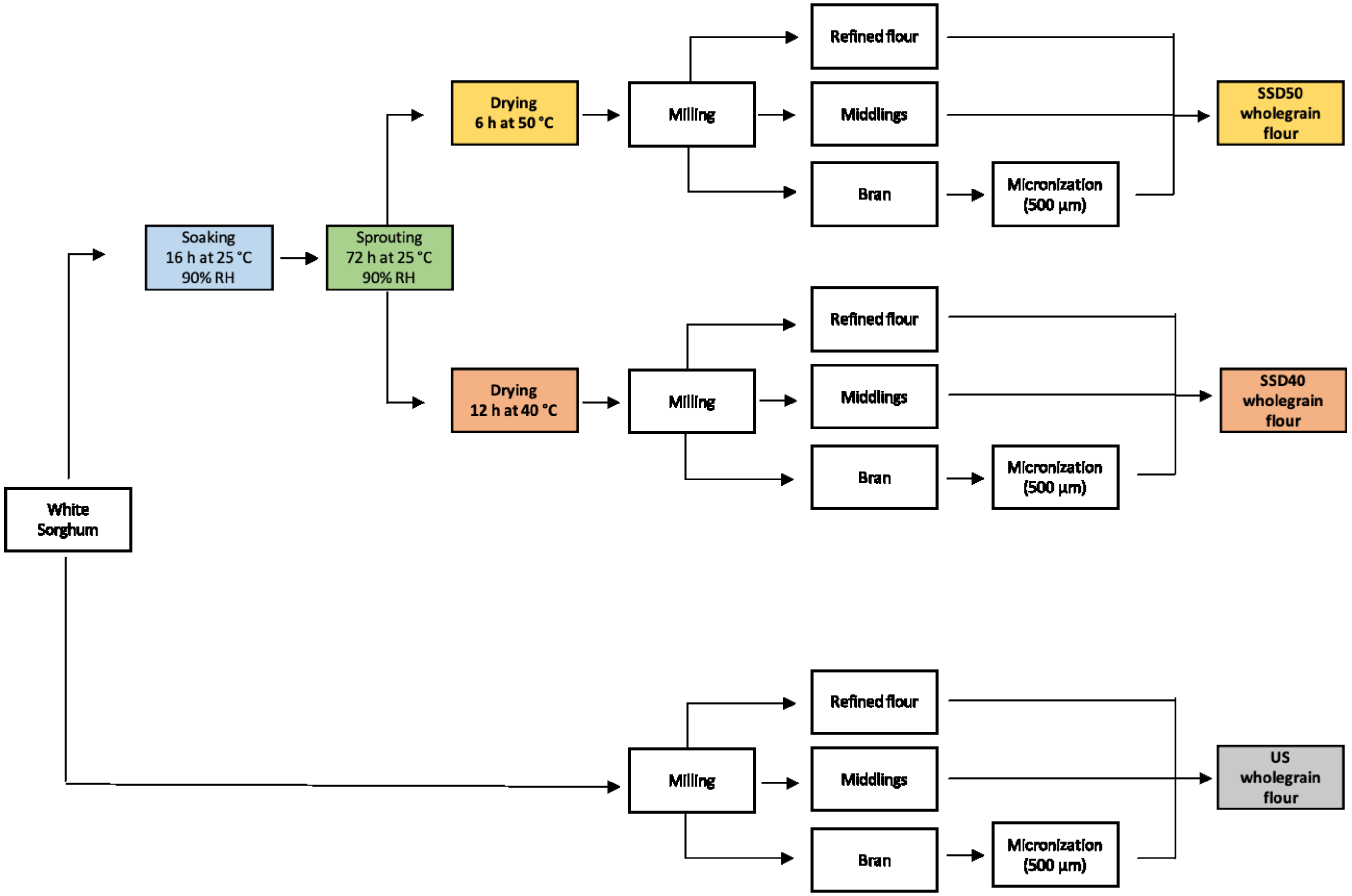

Fig. S1. Schematic representation of sample preparation.

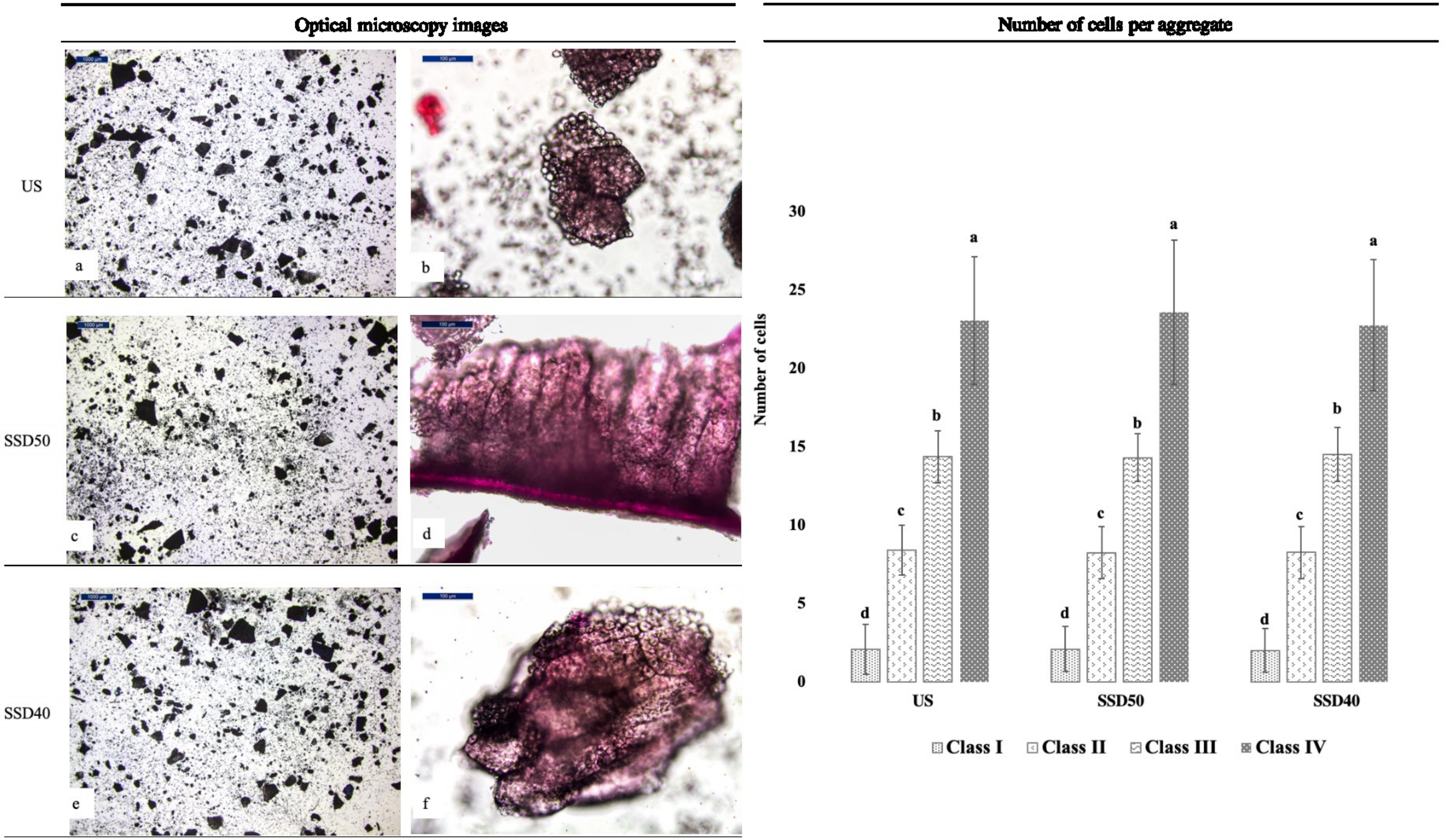

Fig. S2. On the left, morphological observations of sorghum flours acquired with optical microscopy. Cell aggregates morphology (a, c, e; magnified 1.25x) and cells morphology (b, d, f; magnified 20x) in US, SSD50 and SSD40. On the right, number of cells per aggregate. For each sample, different letters mean statistically significant differences among the dimensional classes (one-way ANOVA and Duncan post-hoc) ($p < 0.05$). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

Table S1. Thermal properties of unsprouted and sprouted sorghum flours.

	Starch gelatinization peak				Amylose – lipid complex peak			
	T_{on} (C°)	T_p (C°)	T_{off} (C°)	ΔH (J g^{-1})	T_{on} (C°)	T_p (C°)	T_{off} (C°)	ΔH (J g^{-1})
US	57.53 ± 0.72b	76.65 ± 0.1	91.3 ± 0.22a	2.3 ± 0.13a	94.92 ± 0.71	100.91 ± 0.02a	108 ± 0.28	0.23 ± 0.02b
SSD50	68.68 ± 0.55a	77.14 ± 0.29	85.97 ± 1.34b	1.64 ± 0.01b	95.95 ± 0.36	100.42 ± 0.78a	106.57 ± 0.48	0.22 ± 0.01b
SSD40	68.23 ± 0.18a	76.97 ± 0.16	86.12 ± 0.9b	1.63 ± 0.31b	93.7 ± 1.34	98.67 ± 4.65a	107.52 ± 0.93	0.33 ± 0.01a

Values are expressed as mean ± SD (n=3). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; T_o, onset temperature; T_p, peak temperature; T_{off}, offset temperature; ΔH, gelatinization enthalpy.

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Chapter 2

Drying after sprouting as a potential way to improve the micronutrient content, *in vitro* bioavailability and antioxidant activity of sorghum flour (*Sorghum bicolor* [L.] Moench)

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Author contribution: M.M.: data elaboration and discussion, conceptualization, manuscript writing.

Abstract

Sprouting is proved to be a cost-effective technology to enhance the nutritional value of cereals by promoting structural, chemical and biochemical changes in the grain. Similarly, modulating drying conditions after sorghum sprouting may be an effective, sustainable technology to increase nutritional value of flours. In this work, the effect of sprouting and drying conditions (40 °C for 12 h vs. 50 °C for 6 h) on micronutrient profile of derived flours was assessed.

Overall, sprouting process allowed a decrease in phytic acid, an increase in vitamins, mineral and total polyphenols content along with their soluble and bioaccessible fractions, with a positive impact on the antioxidant potential of wholegrain sorghum flour. In particular, the combination of a lower temperature (40 °C) and longer time (12 h) in the drying treatment caused a lower vitamin thermal degradation and prolonged metabolic and enzymatic pathways involved in soluble phenolic compound biosynthesis and transformation promoted by the sprouting, increasing the soluble and bioaccessible polyphenols and the free radical scavenging activity of the flour.

Sprouting treatment seemed to offer a positive sustainable opportunity for improving the micronutrient profile of the derived flours. Specifically, the flour obtained by the grain undergone to a lower temperature and longer time drying treatment should be taken into consideration as a food ingredient that can potentially improve the nutritional profile of final products.

Keywords

Germination, sorghum, bioactive compounds, antioxidant activity, phytic acid

Abbreviations

US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; d.b., dry basis; MC, moisture content; d.b., dry basis; DPPH•, 2,2-diphenyl-1-picrylhydrazyl radical; AR, antiradical activity; HPLC-DAD/FL, High-Performance Liquid Chromatography coupled with a Diode-Array or Fluorescence Detectors; LC-MS/M, Liquid Chromatography coupled to tandem mass spectrometry; PA, phytic acid; HPLC-UV/Vis, high-performance liquid chromatography coupled with a UV–visible detector; ANOVA, analysis of variance; GAE, gallic acid equivalents; IP/SP, insoluble/soluble polyphenols average ratio.

1. Introduction

In the literature, sprouting has been reported as an inexpensive, cost-effective technology to enhance the nutritional value of cereals by promoting structural, chemical and biochemical changes in the grain. By and large, the enzymatic hydrolytic activity activated during grain sprouting results in a degradation of starch and non-starch polysaccharides and proteins, leading to reduced sugars, peptides and amino acids with unavoidable impacts on nutritional and technological functionality of derived flours. Additionally, the metabolic and enzymatic pathways activated during sprouting synthetize bioactive components, such as vitamins, minerals and phenolic compounds, and also increase their availability by reducing antinutritional factors (Singh, Rehal, Kaur, & Jyot, 2015; Lemmens et al., 2019). An accumulation of soluble and low molecular weight phenolics enhances the antioxidant capacity of flour with potential positive effects on health (Wang, He, & Chen, 2014). Among the cereals, sorghum growing deserves interest because of its industrial application to develop alternative healthy foods in developed countries, as well as for its great importance in terms of nutritional significance and agronomic advantages in low-income countries in the semi-arid tropics (Xiong, Zhang, Warner, & Fang, 2019). In these countries, sprouting has been traditionally empirically performed at a household level and its effects on the nutritional profile improvement (Singh et al., 2015) of derived products may be of great interest.

The effect of sprouting on micronutrients sorghum's profile has been previously investigated. For example, an increase in phytase activity and a decrease in phytates after sprouting has been reported in the literature, affecting mineral content and *in vitro* bioavailability (Egli, Davidsson, Juillerat, Barclay, & Hurrel, 2002; Afify, El-Beltagi, El-Salam, & Omran, 2011). An overall increase in thiamine, riboflavin, niacin, folic acid and pyridoxine content related to biosynthesis processes activated during sprouting has also been reported in the literature (Malleshi & Klopfenstein, 1998; Ochanda, Akoth, Mwasaru, Kagwiria, & Mathooko, 2010). An upsurge in total polyphenol content has also been documented (Donkor et al., 2012). Lastly, decreases in iron and zinc content due to leaching into steeping water, coupled with an increase in their *in vitro* bioavailability related to tannins and phytates reduction during sprouting, have been noted by Afify et al. (2011).

Although the sprouting of sorghum has been investigated in detail over the years, the effect of subsequent drying on bioactive compounds and antioxidant activity of sorghum flour has not been explored to the same extent.

In a previous work (Section B, Chapter 1), significant changes in the nutritional and functional properties of sorghum protein and starch components as a function of different post-sprouting drying conditions have been reported in flours. Given that the temperature-time combination is one of the key parameters when it comes to both metabolic pathway and enzymatic process control (Muralikrishna, & Nirmala, 2005; Gan et al., 2017), and to heat-labile micronutrient retention in cereal sprouts (Lemmens et al., 2019), the drying treatment might affect the derived flour's bioactive components.

Thus, in this work, the effect of drying conditions after sprouting on micronutrient profile of derived flours was assessed. Different time/temperature combinations (40 °C for 12 h *vs.* 50 °C for 6 h) of the drying treatment after sprouting were chosen, and their effects on polyphenol content and *in vitro* bioavailability and antioxidant activity, vitamins, phytic acid and minerals, of the derived flours were investigated.

2. Materials and methods

2.1 Sample preparation

Commercial white sorghum (*Sorghum bicolour* [L.] Moench) grain was soaked in water (kernels:water ratio of 1:2) for 16 h at 25 °C and 90% relative humidity and sprouted for 72 h in an industrial sprouting plant (Bühler AG, Uzwil, Switzerland) under controlled conditions of temperature and humidity, as described in Section B, Chapter 1. Two temperatures were chosen for the subsequent drying treatments: 50 °C, a temperature frequently used in industrial sprouting processes, and 40 °C, a temperature aimed to simulate the sun-drying temperatures in some African countries, where sprouting process is traditionally performed at a domestic level. Therefore, the sprouted kernels were divided into two aliquots and subjected to the following drying treatments: one aliquot was dried for 6 h at 50 °C (SSD50), while the other one aliquot was dried for 12 h at 40 °C (SSD40). An equal flour final moisture content (12.68±0.03%) was obtained for both the processes.

Unsprouted (US) and sprouted sorghum kernels were milled by means of a lab-scale mill (Labormill, BONA, Monza, Italy) to obtain refined flour, middlings, and bran. Any rootlets among the sprouted seeds were not discarded before milling. After bran micronization (500 µm), the fractions obtained were reconstituted to wholegrain sorghum flour, characterized by the following particle mass distribution: ≈ 23% particle size > 300 µm; ≈ 30% particle size between 300 and 200 µm, ≈ 27% between 200 and 100 µm and ≈ 20% particle size < 100 µm. The flour samples were stored at 4 °C until their use for analysis.

Proximate compositions of flours expressed as % (g/100 g dry basis (d.b.)) were the following:

- US: carbohydrates 85. ± 0.12%; protein 11.27 ± 0.31%; fat 3.71 ± 0.03%; ash: 1.35 ± 0.02%; moisture 11.31 ± 0.15% wet basis (w.b.);
- SSD50: carbohydrates 84.48 ± 0.53%; protein 10.98 ± 0.48%; fat 3.25 ± 0.00%; ash 1.30 ± 0.00%; moisture 12.69 ± 0.04% w.b.;
- SSD40: carbohydrates 83.63 ± 0.07%; protein 11.97 ± 0.00%; fat 3.07 ± 0.02%; ash 1.33 ± 0.00; moisture 12.67 ± 0.03% w.b.;

2.2 Determination of polyphenol fractions and free radical scavenging activity

Soluble, insoluble and bio-accessible phenolic fractions and antioxidant activity were determined following the procedures described by Conte et al. (2020). Specifically, soluble and insoluble phenolic fractions were measured as follows: firstly, 1 g of flour was extracted twice using 4 mL of a hydrochloric acid/methanol/water (1/80/10, v/v) solution for 2 h at room temperature under constant agitation. The obtained supernatants were centrifuged (1578 g (3500 rpm), 10 min), collected, filtered and used for the determination of the soluble phenolic fraction; after that, the sample residues were digested in a shaking water bath at 85 °C for 20 h using 5 mL of methanol/concentrated sulphuric acid (10:1, v/v) and used for the determination of the insoluble phenolic fraction. The obtained extracts, with a proper dilution, were spectrophotometrically (Spectrophotometer mod. 8453, Hewlett–Packard, Palo Alto, California) analysed at 750 nm using the Folin-Ciocalteau reagent (Singleton, Orthofer, & Lamuela-Raventós, 1998).

The bioaccessible polyphenol fraction was measured by subjecting flours to an *in vitro* enzymatic digestion, which simulates the conditions in the gastrointestinal tract, slightly modifying the procedure previously described by Glahn, Lee, Yeung, Goldman, & Miller (1998).

For all the polyphenol fractions, calibration curves were made using gallic acid as standard. All the determinations were carried out in duplicate and the results were expressed as mg of gallic acid equivalent/100 g of flour on dry basis (d.b).

The free radical scavenging activity of the flours was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as previously reported by Collar, Jiménez, Conte, & Fadda (2014). The absorbance was read at 515 nm and the extent of the radical reduction was evaluated over a 60 min reaction period. The test was performed in duplicate and plots of μMol DPPH vs. time (min.) were drawn. The antiradical activity (AR) was calculated as following [Equation 1]:

$$\mathrm{AR} = \left[\left(\mathrm{DPPH_{initial}} - \mathrm{DPPH_{plateau}}\right) \times 100\right] / \mathrm{DPPH_{initial}} \qquad [1]$$

2.3 Vitamin content

- **Water-soluble vitamins determination**

Determination of thiamine (B1), riboflavin (B2), niacin (PP), pantothenic acid (B5), pyridoxine (B6), biotin (B8) and folate (B9), was carried out following AOAC Official Methods 970.65 and 942.23 (AOAC, 2005) and the UNI EN 14152:2004 and 14663:2006 procedures. In brief, vitamins were released from the sorghum flours by acid and enzymatic hydrolysis and/or extraction in a weakly basic environment and in the presence of preservatives in an ultrasonic water bath, followed by acetonitrile addition and acidification. The extracts obtained were analysed by High-Performance Liquid Chromatography coupled with a Diode-Array or Fluorescence Detectors (HPLC-DAD/FL), and/or Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS), depending on the analytes being researched and their estimated quantities.

- **Determination of vitamin E content**

The samples were subjected to acidic hydrolysis and extraction with petroleum ether. The extracts obtained were analysed by HPLC-DAD/FL, adapting the method described by Commission Directive 2000/45/EC of 6 July 2000 and the AOAC Official Analysis Methods 972.31 (AOAC, 2000) and 992.03 (AOAC, 2012). All determinations were carried out in duplicate. Results were expressed as mg/100 g d.b.

2.4 Phytic acid (PA) determination

Phytic acid was determined following the method described by Oberleas, & Harland (2007). In brief, after acid hydrolysis, the PA was determined by high-performance liquid chromatography coupled with a UV-visible detector (HPLC-UV/Vis), using an external standard curve (0.1–7.5 g L^{-1}) for quantification. Analyses were performed in triplicate and the results were expressed as g/100 g d.b.

2.5 Mineral content and *in vitro* bioavailability

Analyses on both mineral composition and *in vitro* bioavailability were carried out using Flame Atomic Absorption Spectroscopy (AAnalyst^{TM} 200, PerkinElmer, CT, USA) in line with the AOAC Official Analysis Methods (AOAC, 1997).

Briefly, for the determination of the mineral content, 0.5 g of ground sample were weighed into vessels and mineralized with 8 mL of nitric acid and 2 mL of hydrogen peroxide (30%) for 40 min at 200 °C in a microwave digestion system (Ethos Easy, Milestone Srl, BG, Italy). After cooling, the mineralized samples were filtered (Whatman filter paper 8 µm, Fisher Scientific, Massachusetts, USA) into 50 mL volumetric flasks and filled to the mark with ultrapure water. The determination of the bioavailable mineral fraction was obtained by applying this same procedure to the *in vitro* digestive enzymatic extracts also used for the determination of the bioaccessible polyphenol fraction. Quantification of the macroelements (Ca, Mg, Na, K) and microelements (Mn, Cu, Zn, Ni, Fe) was calculated using calibration curves with $R^2 > 0.998$. Results were expressed as mg/100 g d.b. of three replicates.

2.6 Statistical analyses

To verify significant differences between samples, the data obtained were statistically analysed by performing one-way analysis of variance (ANOVA) followed by Duncan's *post-hoc* test 0.05 significance level, using SPSS Software Version 25.0 (SPSS Inc., IL, USA). Pearson correlation analysis for relationships between antiradical activity and polyphenols fractions, and antiradical activity and vitamin E was also used.

3. Results and discussion

3.1 Determination of polyphenol fractions and antioxidant activity

Data on total polyphenols and soluble, insoluble and bioaccessible fractions are presented in Table 1.

Table 1. Polyphenol fractions of sorghum flours. Values are expressed as mean ± SD (n=2). Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test. $p \leq 0.05$). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; GAE, gallic acid equivalent; d.b., dry basis; IP/PS, insoluble polyphenols/soluble polyphenols ratio.

Sample	Polyphenol fractions (mg GAE/100 g d.b.)					
	Soluble	Insoluble	IP/SP	Total	Bioaccessible	Δ bioaccessibility (%)
US	197.33 ± 0.13c	451.73 ± 2.30a	2.29	650.70 ± 4.47b	461.84 ± 2.64c	-
SSD50	404.20 ± 0.70b	517.49 ± 27.22a	1.28	921.68 ± 27.92a	647.43 ± 5.62b	40
SSD40	410.93 ± 0.69a	505.70 ± 8.37a	1.23	916.63 ± 7.69a	667.88 ± 1.09a	45

The total amount of polyphenols found in US was 650.70 ± 4.47 mg of GAE/100 g d.b., in line with the ranges previously reported (Dicko, Gruppen, Traoré, van Berkel, & Voragen, 2005; Salazar-López, González-Aguilar, Rouzaud-Sández, & Robles-Sánchez, 2018; Xiong et al., 2019). As expected, sprouting process increased the total polyphenol content (≈41%), with no significant differences as a function of the drying condition (SSD50 vs SSD40). Many previous studies have proved sprouting as a way to increase the level of total phenolic compounds in edible seeds−including sorghum−with different impacts on their soluble and insoluble fractions (Gan et al., 2017; Lemmens et al., 2019; Salazar-López et al., 2018). Most studies have demonstrated that sprouting can accumulate soluble phenolics in seeds, which may have been *de novo* synthetized in the intracellular endoplasmic reticulum and stored in vacuoles, or they may have been released from the bound fractions by hydrolytic enzymes during the germination process. Indeed, the bound fractions result by a conjugation of soluble phenolics with cell wall macromolecules thereby contributing to cell wall formation (Gan et al., 2017).

In our study, the soluble and insoluble fractions in US represented ~30% and ~69% of the total phenolic content, respectively. Data reported in Table 1 showed an increase in soluble polyphenols and no significant differences in the insoluble polyphenols upon sprouting. However, comparing data expressed on the total polyphenol content, it was noticed that sprouting not only increased the soluble fractions, but also decreased the insoluble ones (~45% and ~55% of the total polyphenols, respectively). Furthermore, a decrease in the insoluble/soluble polyphenol average ratio (IP/SP, Table 1) was observed upon sprouting, which was related to the soluble fraction increase, since the insoluble fraction did not differ among samples. Such a decrease in the IP/SP ratio indicated a significant increase in the extractable polyphenols, which may result in a nutritional enrichment of the final product (Conte et al., 2020).

The % bioaccessible polyphenols was also quantified and it represented 70% of the total in US. Moreover, both sprouted samples exhibited an increment in polyphenol bioaccessibility with respect to the US, with the SSD40 showing the highest value (40% and 45%, for SSD50 and SSD40, respectively) (Table 1).

Given that free and some soluble conjugated phenolic acids have been found to be easily absorbed by the human intestine, while most of the insoluble bound phenolic compounds have very low bioaccessibility and bioavailability (Wang et al., 2014), the decrease in IP/SP ratio and the increase in soluble and bioaccessible polyphenols confirm the effect of sprouting in increasing the polyphenol bioaccessibility with potential health promoting properties. Moreover, it is important to underline that these positive changes were more evident in SSD40 sample where the low temperature applied in the drying treatment may have prolonged the metabolic pathways and enzymatic processes involved in soluble phenolic compound biosynthesis and transformation promoted by the sprouting (Gan et al., 2017).

It is a well-established fact that several phenolic compounds, including phenolic acids and flavonoids can exhibit a strong antioxidant potential thanks to their ability to neutralize free radicals (Conte et al., 2020).

The free radical scavenging activity of sorghum flours was measured by testing the flour extracts' ability to quench the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical. Fig.1 showed the remaining unreacted µMol of DPPH• until a plateau (steady state) was reached.

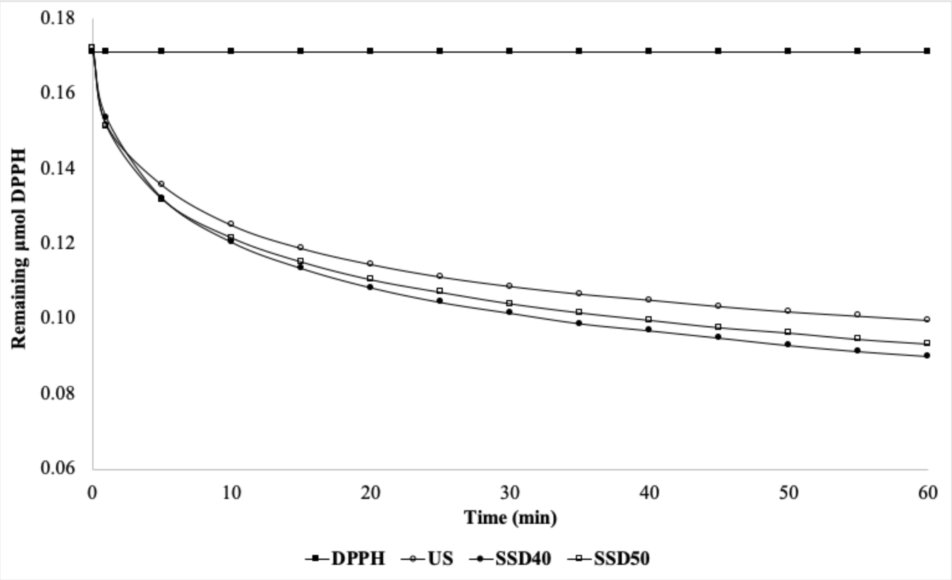

Fig. 1. Time evolution of the DPPH curves in methanol of organic extracts from sorghum flours. DPPH (■). US (○). SSD40 (●). SSD50 (□). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h.

The results of anti-radical activity (AR) in the sorghum flours (Table 2) revealed that all the flours showed significant scavenging activity against DPPH• radical, with US showing an AR of about 42%.

Table 2. antioxidant activity of sorghum flours. Values are expressed as mean ± SD (n=2). Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test. $p \leq 0.05$). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h

Sample	Anti-radical activity (%, AR)	
		Remaining µmol DPPH at steady state
US	42.13 ± 0.3c	0.100 ± 0.001a
SSD50	45.77 ± 0.2b	0.093 ± 0.002a
SSD40	47.44 ± 0.1a	0.090 ± 0.000a

As expected, and in agreement with the literature (Gan et al., 2017; Lemmens et al., 2019), a significant ($p \leq 0.05$) increase in AR was recorded upon sprouting. Specifically, in comparison to the US, the sprouting treatments applied to SSD50 and SSD40 increased AR by up to 9% and 13%, respectively, showing the highest value in USSD40 sample. This enhancement in free radical scavenging activity is consistent with the increase in both total polyphenols and antioxidant vitamins (i.e., vit. E, as discussed below) (Dicko et al., 2005) in sprouted samples, particularly in SSD40.

Pearson correlations were used to analyse relation between antiradical activity and phenolics fractions. Values of correlation coefficients (r) revealed that higher value of antiradical activity corresponded to larger amounts of soluble (r=0.96; $p < 0.01$), insoluble (r=0.83; $p < 0.05$), total (r=0.94; $p < 0.01$) and bioaccessible (r=0.97; $p < 0.01$) polyphenols fractions.

These results demonstrated that sprouting could provide a source of natural antioxidative compounds with potent beneficial health effects and that the drying conditions could also be a way to further modulate it.

3.2 Vitamin content

The B-vitamins (B1, B2, PP, B5, B6, B8, B9) and Vitamin E content of the flours is reported in Table 3.

Table 3 Vitamins content of unsprouted and sprouted sorghum flours. Values are expressed on dry basis as mean ± SD (n=3). Values followed by different letters in each raw are significantly different (one-way ANOVA with Duncan's post-hoc test. $p \leq 0.05$). US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; d.b., dry basis.

	US	SSD50	SSD40
Thiamine (B1, mg/100 g d.b.)	0.31 ± 0.02a	0.33 ± 0.01a	0.32 ± 0.00a
Riboflavin (B2, mg/100 g d.b.)	0.07 ± 0.00c	0.13 ± 0.01b	0.14 ± 0.00a
Niacin (PP, mg/100 g d.b.)	5.87 ± 0.62b	7.35 ± 0.25a	5.93 ± 0.36b
Pantothenic Acid (B5, mg/100 g d.b.)	2.38 ± 0.14b	2.87 ± 0.13a	3.04 ± 0.02a
Pyridoxine (B6, mg/100 g d.b.)	0.31 ± 0.01c	0.35 ± 0.02b	0.39 ± 0.01a
Biotin (B8, µg/100 g d.b.)	16.63 ± 1.11b	16.73 ± 1.12b	18.76 ± 0.00a
Folate (B9, µg/100 g d.b.)	23.28 ± 20b	24.53 ± 1.84b	28.69 ± 2.21a
Vit. E (mg/100 g d.b.)	0.69 ± 0.05c	1.22 ± 0.11b	2.21 ± 0.03a

Data found for US were in line with the literature (Malleshi, & Klopfenstein, 1998; Ochanda et al., 2010) and the international databases (Lukmanji, Hertzmark, Mlingi, Assey, Ndossi, & Fawzi, 2008; USDA 2008). As expected, sprouting increased most of the vitamins (Table 3) due to the biosynthesis processes activated to support seedling development and growth (Lemmens et al., 2019). A greater rise in vitamin contents was found for SSD40 than for SSD50 (Table 3). Specifically, compared to US, SSD40 showed a significative ($p \leq 0.05$) increase in vits. B2, B5, B6, B8, B9 and E of around 109%, 28%, 26%, 13%, 23% and 219% respectively, while vit. B1 and PP contents did not change significantly. Similarly, in comparison to US, SSD50 showed a significative ($p \leq 0.05$) raise in vits. B2, PP, B5, B6 and E of around 87%, 25%, 20%, 14% and 76%, respectively, while no significative differences were found in the contents of vits. B1, B8 and B9.

Furthermore, as expected, Pearson correlation revealed a strong positive correlation between vitamin E content and flour's free-radical scavenging activity (r=0.92; $p < 0.01$), thus confirming the beneficial role of sprouting in providing natural antioxidative compounds.

From data shown in Table 3, it may be assumed that the drying treatment performed on SSD40, in comparison to that performed on SSD50, determined a lower loss of vits. B2, B6, B8, and folate, a higher loss of vit. PP, and no differences in vit. B1 and B5 content.

The significant fluctuations in thiamine content throughout the steeping and sprouting phases and its high heat-lability documented in the literature (Hucker, Wakeling, & Vriesekoop, 2012; Lemmens et al., 2019) may explain the comparable vit. B1 values among the three samples.

Overall, the two time/temperature combinations used in the drying treatment affected the vitamins content of the samples in different ways. In this case, the treatment at lower temperature and longer time caused a minor loss of most vitamins. Conceivably, the lower amount of certain vitamins found in the SSD50 treatment may have been due to the higher temperature used, thereby reaching their degradation temperatures range (e.g. vit. B2, whose degradation temperature range starts at 50 °C) (Sheraz, Kazi, Ahmed, Anwar, & Ahmad, 2014).

3.3 Phytic acid (PA)

Phytic acid (PA, myo-inositol-1,2,3,4,5,6-hexakisphosphate) is a naturally occurring compound of plant food commonly considered as anti-nutritional dietary factor. Indeed, PA shows a potential ability to chelate positively charged cations, such as those of iron (Fe), zinc (Zn), calcium (Ca), manganese (Mn), magnesium (Mg), and copper (Cu), thereby affecting mineral bioavailability and bioaccessibility (Erba, Manini, Meroni, & Casiraghi, 2017; Lemmens et al., 2019). It is indeed well documented that sprouting is a highly effective process for reducing phytic acid in cereal grains (Omary, Fong, Rothschild, & Finney, 2012; Lemmens et al., 2019). The dephytinization effect consequent to sprouting is related to the *de novo* synthesis and activation of endogenous phytase and phosphorylases, which hydrolyse phytate to release bound phosphate, mineral elements, and myoinositol available for plant growth and development, thus increasing mineral bioavailability and bioaccessibility (Egli et al., 2002).

The PA content of US flour was found to be 1.4 ± 0.05 g/100 g d.b., which was within the range previously identified in the literature (Makokha, Oniang'o, Njoroge, & Kamar, 2002). As expected, a significant ($p \leq 0.05$) decrease in PA occurred upon sprouting. The PA detected for SSD50 and SSD40 was 1.14 ± 0.05 and 1.24 ± 0.04 g/100 g d.b, respectively, 20% and 12% lower than the value recorded for US. The PA contents of the two sprouted flours did not significantly differ, indicating that these changes were influenced by sprouting and not by the subsequent drying treatment. Cereal phytase optimum temperatures range from 37 to 55 °C (Lemmens et al., 2019). It is therefore reasonable to assume that the endogenous phytase may be active during the initial drying phases thereby promoting the phytate breakdown. However, the not significant differences in PA content found in the SSD40 and SSD50 samples demonstrate that the different drying conditions (time, temperature) adopted in this study were probably equally effective in terms of their enzyme inhibition.

3.4 Mineral content and *in vitro* bioavailability

The mineral composition of sorghum flours is reported in Table 4.

Table 4 Mineral content and *in vitro* bioavailability (mg/100 g d.b.) of sorghum flours. Values are expressed as mean ± SD (n=3). Values followed by different letters in each raw are significantly different (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$). In brackets, the % of bioavailability on the total mineral content. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; n.a., not available data.

	Mineral content (g/100 g d.b.)			*In vitro* mineral bioavailability (g/100 g d.b.)		
	US	SSD50	SSD40	US	SSD50	SSD40
Macroelements						
Ca	3.85 ± 0.05c	7.47 ± 0.27a	5.01 ± 0.27b	3.61 ± 0.05a (93.92)	3.46 ± 0.02a (46.27)	3.46 ± 0.24a (69.22)
Mg	94.78 ± 2.23a	94.8 ± 0a	97.84 ± 0.04a	79.8 ± 0.03b (84.2)	84.76 ± 3.64ab (89.41)	88.27 ± 0.03a (90.22)
Na	0.56 ± 0a	0.56 ± 0a	0.57 ± 0.01a	n.a.	n.a.	n.a.
K	319.19 ± 1.28a	259.85 ± 1.36c	283.68 ± 0b	190.64 ± 3.54a (59.73)	182.9 ± 10.93a (70.39)	163.3 ± 3.56b (57.57)
Microelements						
Mn	1.05 ± 0.01c	1.38 ± 0a	1.21 ± 0.08b	0.6 ± 0.02b (57.32)	0.8 ± 0.01a (57.97)	0.72 ± 0.05a (59.66)
Cu	0.5 ± 0.03a	0.55 ± 0.01a	0.54 ± 0a	0.29 ± 0.02a (58.17)	0.27 ± 0a (48.85)	0.22 ± 0.04a (40.64)
Zn	2.66 ± 0.01a	2.69 ± 0.08a	2.77 ± 0.07a	1.06 ± 0.04b (40.02)	1.16 ± 0a (43.18)	1.1 ± 0.04ab (39.83)
Ni	0.09 ± 0b	0.13 ± 0.01a	0.14 ± 0.01a	n.a.	n.a.	n.a.
Fe	0.47 ± 0.01c	0.62 ± 0a	0.52 ± 0.02b	0.17 ± 0b (36.36)	0.15 ± 0.01b (24.48)	0.19 ± 0.01a (36.82)

The mineral contents of the US sample are overall consistent with literature (Paiva, Queiroz, Simeone, Schaffert, de Oliveira, & da Silva, 2017) and international databases (USDA, 2008).

Mg, Na, Cu and Zn contents were not affected by the entire sprouting process in either SSD40 or SSD50 conditions. Concerning the other macroelements analysed, compared to US, SSD50 treatment conditions caused a 94% increase and a 19% decrease in Ca and K contents, respectively. On the other hand, the SSD40 treatment conditions caused a 30% increase and a 11% decrease in the aforementioned minerals, respectively. The observed loss of K in both sprouted samples could be due to leaching in the steeping water during the sprouting phase (Lemmens et al., 2019).

Overall, SSD40 recorded a 33% lower content in Ca and a 9% higher content in K compared to SSD50.

Concerning microelements, both the sprouted samples had significative ($p \leq 0.05$) higher Mn, Ni and Fe contents than US. Specifically, SSD50 showed a ~30% increase in all the three minerals, while SSD40 showed a rise of around 16, 42 and 11%, respectively. Furthermore, the treatment used in SSD40 determined a 7% higher content of Ni and a 12% and 16% lower content in Mn and Fe than that used in SSD50.

Inconsistent results were found in literature about the effect of sorghum sprouting on mineral composition. An overall increase of mineral in three sorghum cultivars was reported by Mohamed Nour and colleagues (Mohamed Nour, Mohamed Ahmed, Babiker, & Yagoub, 2010) after germination for up to 72 hr, while studies by Afify et al. (2011) and Elkhier, & Hamid (2008) reported overall decrease in minerals in sorghum cultivars germinated for 3-7 days. In our study, sprouting caused an overall increase in Ca, Mn, Ni and Fe and a decrease in K content compared to the unsprouted sample, with SSD50 flour showing the major changes on these minerals.

Minerals *in vitro* bioavailability of sorghum flours (mg/100 g d.b.) is reported in Table 4. Sprouting process differently affected the minerals bioavailability. Compared to US, the bioavailability of Ca and Cu did not change as a consequence of sprouting, the Mn one significantly ($p \leq 0.05$) increased in both the sprouted samples, while the increase in Mg bioavailability resulted statistically significant only for SSD40. Otherwise, K, Zn and Fe bioavailability changed in a discordant way in the two sprouted samples compared to US, thus not allowing to properly assess the effect of the entire sprouting process on these minerals. Furthermore, comparing data obtained by the two sprouted samples (Table 3), it was not possible to outline a general effect of the two sprouting treatments on the % mineral bioavailability, since Ca, Mg, Mn and Fe *in vitro* bioavailability was higher in SSD40 than in SSD50, while for the other minerals it resulted lower.

A general increase in mineral *in vitro* bioavailability in most varieties and cultivars of sorghum was previously observed upon sprouting (Idris, Hassan, Babiker, & El Tinay, 2005; Afify et al., 2011).

In our study, obtained data on minerals highlighted a general increase in their contents upon sprouting, especially for SSD50 sample, but an irregular trend was observed for their *in vitro* bioavailability. This behaviour may be caused by the increase in polyphenols and the simultaneous reduction of PA (as discussed before), which are recognised to be antinutrients due to their ability to bind minerals, making them unavailable (Omary et al., 2012). Therefore, an increase in the content of the former in association with a reduction in the content of the latter, may have promoted contrasting effects on minerals bioavailability.

Conclusion

This work attempted to evaluate for the first time the effect of drying treatments after sorghum sprouting on the micronutrient profile and antioxidant capacity of the derived flours. Overall, both the processes applied to sorghum seemed to offer a positive sustainable opportunity for improving the micronutrient profile of the derived flours, allowing a decrease in phytic acid, an increase in vitamin, mineral and total polyphenol content along with their soluble and bioaccessible fractions, with a positive impact on the antioxidant potential of wholegrain sorghum flour. However, the combination of a lower temperature (40 °C) and longer time (12 h) in the drying treatment caused a lower vitamin thermal degradation in the flour and prolonged metabolic and enzymatic pathways involved in soluble phenolic compound biosynthesis and transformation promoted by the sprouting, increasing the soluble and bioaccessible polyphenols and the free radical scavenging activity of the flour. Consequently, the flour obtained by lower temperature and longer time drying treatment applied to sprouted sorghum, should be taken into consideration as a food ingredient that can potentially improve the nutritional profile of final products.

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Chapter 3

Insight into molecular and rheological properties of sprouted sorghum flour

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Abstract

This work investigated the effect of sprouting and drying post-sprouting on technological functionalities of sorghum flour as probed by Low-resolution proton nuclear magnetic resonance (^{1}H NMR) and Dynamic Mechanical Analysis (DMA). Multivariate statistics were used to assess the effect of flour (from sprouted and unsprouted sorghum, and wholewheat) and hydration level on flour-water systems molecular and viscoelastic properties. Overall, sorghum-based systems showed greater molecular mobility explaining poorer viscoelastic properties than those obtained from wheat. Sprouting affected the molecular properties of sorghum flour-water systems, while no differences were observed in the two sprouted samples dried in different conditions. However, sprouting did not affect the viscoelastic properties of sorghum-water systems. These results bolster the use of sprouted sorghum in composite flours for the development of sustainable finished products with high nutritional value and satisfactory technological and organoleptic properties.

Keywords

Sorghum, germination, rheological properties, molecular mobilities, water dynamics, multivariate statistics

Abbreviations

LR ^{1}H NMR, Low-Resolution Proton Nuclear Magnetic Resonance; DMA, Dynamic Mechanical Analysis; US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; W, organic wholewheat flour; d. b., dry basis; w. b., wet basis; alveographic parameters – W (J 10^{-4}), baking strength; P/L ratio, curve configuration ratio; P (mm), dough tenacity; L (mm), dough extensibility; SS, flour from sprouted sorghum.

1. Introduction

In the literature, sprouting of sorghum has been confirmed as a sustainable technology which causes structural changes in the grain with an effect on the functionality of its flour. In particular, an improvement of the nutritional and sensory profile and changes in the technological properties in both the grain and its flour have been reported (Singh, Sharma, & Singh, 2017; Lemmens et al., 2019). Sprouting triggers proteases and amylases which break down protein and starch. Protein hydrolysis causes higher solubility, water and oil holding capacity, more foam and emulsion capacity and stability than in unsprouted sorghum flour (Elkhalifa & Bernhardt, 2010; Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011; Singh et al., 2017). In addition, degradation of protein resulted in higher starch hydrolysis by amylases, which worsens the pasting properties of flour when dispersed in excess of water and stirred under a controlled heating/cooling temperature program, and changed the textural properties of hydrolyzed starch (Marengo, Bonomi, Marti, Pagani, Elkhalifa, & Iametti, 2015; Yi, Li, & Ping, 2017).

Recently, it has been reported that also the post-sprouting drying treatment can modulate the starch and protein functionalities of the flour obtained by sprouted sorghum (Marchini et al., 2021). Indeed, a drying treatment performed at lower temperature/longer time (40 °C for 12 h) was demonstrated to extend the enzymatic activities triggered during sprouting than a treatment performed at higher temperature/shorter time (50 °C for 6 h), resulting in an increased protein hydrolysis and water and oil holding capacity, higher starch digestibility, but also in a worsening of flour technological properties (Marchini et al., 2021). In any case, while on the one hand the use of flour from sprouted sorghum is of relevance to increase the nutritional value of finished products, on the other, its technological functionality may hinder the use. Therefore, it is mandatory to assess its feasibility to finished product – making.

Those works that have studied the effects of the sprouting process on the technological properties of sorghum flour have focused above all on the macroscopic properties, e.g., the flours' water holding capacity, water absorption capacity and swelling power, pasting properties, and rheological behaviour through the use of a Mixolab Device (Mahajan, & Gupta, 2015; Elkhalifa, & Bernhardt, 2018).

Besides these, techniques able to investigate the physicochemical properties of flour biopolymers and their interactions with water at molecular scale can be of particular interest, since these interactions are strongly related to the mesoscopic (i.e., viscoelastic) properties of the final product.

In this perspective, Low-resolution Proton Nuclear Magnetic Resonance (LR ^{1}H NMR) is a non-destructive technique that has been proven powerful to study the molecular mobility and dynamics of water and biopolymers in foods (Kirtil, Cikrikci, Mccarthy, & Oztop, 2017). In the literature, many authors have applied LR ^{1}H NMR spectroscopy to cereal-based (mainly wheat and corn) doughs, in order to assign different proton molecular domains to different flour biopolymers and to study their interactions with water (Raun, Wang, Chen, Fulcher, Pesheck, & Chakrabarti, 1999; Bosmans, Lagrain, Deleu, Fierens, Hills, & Delcour, 2012; Lu, & Seetharaman, 2013; Hager, Bosmans, & Delcour, 2014). Dough systems have been studied as a function of moisture content, fiber, temperature and mixing times with the final aim to relate dough proton dynamics to product quality (Assifaoui, Champion, Chiotelli, & Verel, 2006; Doona, & Baik, 2007; Serial et al., 2016;

Hemdane, Jacobs, Bosmans, Verspreet, Delcour, & Courtin, 2017; Xiong, Zhang, Niu, & Zhao, 2017; Parenti, Guerrini, Zanoni, Marchini, Tuccio, & Carini, 2020).

To the best of the authors' knowledge, LR ^{1}H NMR technique have never been applied before to describe proton mobility in sorghum-based systems; its use could reveal disclosure about the effect of grain sprouting on the molecular properties of flour biopolymers. The interaction of water with proteins, starch and fiber established during the mixing phase at molecular level directly influences the dough viscoelasticity, and therefore the final product quality. Viscoelasticity properties of a material may be assessed by Dynamic Mechanical Analysis (DMA). DMA is a versatile, sensitive technique to measure the modulus (stiffness) and damping (energy dissipation) of materials deformed under periodic stress. DMA has been used to measure changes in the structural and intrinsic properties of polymeric materials, to yield information on food's mechanical properties and how these are affected by various processing conditions (Murayama, 1978).

In this perspective, the aim of this work was to investigate the effect of sprouting and drying post-sprouting treatments on the technological functionalities of sorghum flour by means of the study of molecular and rheological properties of sprouted sorghum flour-water systems with LR ^{1}H NMR and DMA techniques.

2. Materials and methods

2.1 Materials

Commercial white sorghum kernels (*Sorghum bicolor* [L.] Moench) were divided into three aliquots. A 10 kg-aliquot (US) was milled using a Bona laboratory mill (Labormill, Monza, MB, Italy), obtaining refined flour, middlings, and bran. After bran micronization (500 µm), the fractions were reconstituted to wholegrain sorghum flour characterized by the following particle mass distribution: ≈ 23% particle size > 300 µm; ≈ 30% particle size between 300 and 200 µm, ≈ 27% between 200 and 100 µm and ≈ 20% particle size < 100 µm.

Other two aliquots (15 kg each) were sprouted in an industrial plant (Bühler AG, Uzwil, Switzerland) as previously prescribed (Section B, Chapter 1). In brief, the following conditions were used: soaking for 16 h (25 °C, 90% relative humidity) and sprouting for 72 h (25 °C, 90% relative humidity). These sprouting conditions were selected based on a preliminary literature review of processing conditions most adopted in sorghum sprouting processes (Dicko, Gruppen, Zouzouho, Traoré, Van Berkel, & Voragen, 2006; Mahajan, & Gupta, 2015; Marengo et al., 2015; Lemmens et al., 2019). Each aliquot underwent to a different drying treatment. Indeed, two temperatures were chosen for the drying treatment (Marchini et al., 2021), i.e., 50 °C, which may be more representative of the conditions adopted in processes performed at an industrial level (Nkama, Gbenyi, & Hamaker, 2015; Marti, Cardone, Nicolodi, Quaglia, & Pagani, 2017), and 40 °C, more representative of the conditions adopted in those contexts where sprouting is traditionally performed at home and the sprouts are dried in the sun (Elkhalifa, & Bernhardt, 2010; Phattanakulkaewmorie, Paseephol, & Moongngarm, 2011). Therefore, one aliquot was dried for 6 h at 50 °C (SSD50), another for 12 h at 40 °C (SSD40) to obtain the same final moisture content for both processes.

Sprouted grains were then milled into wholegrain flours as described for the unsprouted kernels. The rootlets of sprouted seeds were recovered before milling and added to the bran.

Wholewheat flour [W = 240 10^{-4} J and P/L = 0.55 (Molino Grassi S.p.A., Fraore, PR, Italy)] was used as a control (W). Proximate composition of flour samples is reported in Table S1.

2.2 Flour-water systems preparation

Flour-water systems from US, SSD50, SSD40 and W flours at three different moisture contents [MC, 43±0.01, 48±0.02 and 53±0.01% w.b., as measured by weight loss after forced-air oven drying at 105 °C to constant weight (ISCO NSV 9035, ISCO Srl, Milano, MI, Italy)] were prepared by mixing distilled water and flour for 15 min at room temperature in a home bread-maker (Backmeister 68511, UNHOLD, Hockenheim, Germany) employing a "personalized" program (kneading 15 min; proofing 0 min; baking 0 min). The hydration range was defined based on preliminary trials performed by mixing sorghum flour with water at increasing hydration levels. At MC < 43%, the flour was not fully and properly hydrated, resulting in not-workable doughs. At a MC > 53%, the dough resulted excessively sticky. Therefore, 43% and 53% moisture contents were chosen as lower and upper limit for the workability of sorghum flour-based systems. An intermediate moisture content (48%) was also chosen for analyses. The systems obtained were analysed immediately after production. Two batches of each dough were produced.

2.3 Viscoelastic properties

Flour-water systems' viscoelastic properties [Storage modulus (E'), loss modulus (E') and phase angle (tanδ, described as an E"/E' ratio)] of the doughs were measured using a Dynamic Mechanical Analyser (DMA – Q800, TA Instruments, New Castle, DE, USA). The storage modulus (E') represents the energy stored in the elastic structure of the sample, while the loss modulus (E") represents the viscous part or the amount of energy dissipated in the sample. Meanwhile, tanδ (E"/E') indicates the relative degree of energy dissipation or damping of the material (Menard, & Menard, 2002).

After compressing the material under a 5 kg load for 5 min, small cylinders were extracted using a mould of 12.5 mm in diameter and 10 mm thick, and analysed in compression mode (15 mm diameter parallel plate compression clamp) with a frequency sweep test carried out at 25 °C in the 0.2-10 Hz frequency range. Amplitude was set to 0.8 µm, within the linear viscoelastic region preliminary determined with a strain sweep test (amplitude: 0.1-10 µm; frequency: 1 Hz; temperature: 25 °C).

At least five replicate samples were performed for each flour-water system and for each batch. A single frequency (5.6 Hz) was selected to statistically compare the E', E" and tanδ obtained from different samples.

2.4 Proton molecular mobility

^{1}H molecular mobility was studied using a Low-resolution Nuclear Magnetic Resonance (NMR) spectrometer (20 MHz, the MiniSpec, Bruker Biospin, Milan, MI, Italy) working at 25.0 ± 0.1 °C. Approximately 4 g of sample was placed into an NMR tube (10 mm in diameter) and sealed with Parafilm® to avoid water loss during testing. ^{1}H Free Induction Decay (^{1}H FID) and proton transverse relaxation time (^{1}H T_2) experiments were performed to investigate the less and more mobile protons, respectively. ^{1}H FIDs were acquired using a single 90° pulse, followed by a dwell time of 7 μs, a recycle delay of 1 s, a 0.5 ms acquisition window, with 32 scans and 900 data points. The curves were fitted with a two-component model (exponential and gaussian; Le Grand, Cambert, & Mariette, 2007) to obtain quantitative information about the relaxation time and relative abundance of protons belonging to the more rigid and more mobile proton populations detectable within the FID experimental time frame (ranging from 7 to 500 μs). The fitting was performed using SigmaPlot v.6 software (Systat Software Inc., San Jose, CA, USA) according to the following equation [1]:

$$f(t) = y_0 + A * e^{\left(-\frac{t}{TA}\right)} + B * e^{\left(-\frac{t}{TB}\right)^2} \qquad [1]$$

where y_0 is the FID decay offset, A and B are the intensities of each relaxation component, and TA and TB are the apparent relaxation times.

^{1}H T_2 relaxation time was measured using a Carr-Purcel-Meiboom-Gill (CPMG) pulse sequence with a recycle delay of 1s, interpulse spacing of 0.04 ms, with 2,500 data points and 32 scans. ^{1}H T_2 curves were analysed as quasi-continuous distributions of relaxation times using UpenWin software (University of Bologna, Italy). Default values for all UPEN parameters were used except for LoXtrap parameter, which was set = 1 to avoid extrapolation of relaxation times shorter than the first experimental point. ^{1}H T_2 CPMG relaxation decays were also fitted with a discrete exponential model (Sigmaplot, v.6, Systat Software Inc., San Jose, CA, USA) to obtain relaxation times and proton population abundances, according to equation [2]:

$$F(x)=y_0 + ae^{-bx} + ce^{-dx} + ge^{-hx} + ie^{-fx} \qquad [2]$$

where y_0 is intercept, a, c, g and i, the relative abundances (%) of population C, D, E and F (%PopC, %PopD, %PopE, %PopF), b, d, h and f, the relaxation times (ms) of PopC, PopD, PopE, PopF, respectively (T_{2C}, T_{2D}, T_{2E} and T_{2F}).

Two dough batches were prepared for each sample. Two tubes were analysed for each system and six ^{1}H FID and ^{1}H T_2 experimental curves were acquired for each tube for a total of twenty-four curves for each sample.

2.5 Statistical analyses

NMR and DMA data were analysed using SIMCA® Software (Version 16.0.1, Umetrics Suite, Sartorius Stedim Biotech, Umeå, Sweden) for multivariate statistical data analysis by applying unsupervised Principal Component Analysis (PCA) and its supervised extension Orthogonal Projection to Latent Structures - Discriminant Analysis (OPLS-DA).

The dataset was composed by parameters obtained for each of the two batches produced for each type of flour (4: US, SSD40, SSD50 and W) at each hydration level (3: 43, 48 and 53%), resulting in 24 items (for US: US43a and US43b, US48a and US48b, US53a and US53b; for SSD40: SSD4043a and SSD4043b, SSD4048a and SSD4048b, SSD4053a and SSD4053b; for SSD50: SSD5043a and SSD5043b, SSD5048a and SSD5048b, SSD5053a and SSD5053b; for W: W43a and W43b, W48a and W48b, W53a and W53b).

Concerning rheological measurements, the dataset included the mean values calculated for E', E" and tanδ recorded at a single frequency of 5.6 Hz (Table S3). Instead, molecular parameters included in the dataset were the relative abundances (%PopA and %PopB) and the relaxation times ($FIDT_A$ and $FIDT_B$) of the two proton populations detected in the FID experiment, and similarly the relative abundances (%PopC, %PopD, %PopE and %PopF) and the relaxation times (T_2C, T_2D, T_2E and T_2F) of the four proton populations obtained from the CPMG pulse sequence (Table S2).

Data were mean-centered and scaled to Unit Variance (UV - Autoscaling) as data pre-treatment. OPLS is a multivariate data analysis technique derived from the PLS-regression method and it is useful to improve the interpretability of built PLS models. OPLS can be used as supervised Discriminant Analysis method by assigning categories for each group of observations (e.g., samples) in order to guide the model in finding the best way to discriminate groups against each other and therefore to discover the most important variables for each group. Unlike PLS, OPLS removes structured noise by dividing the information within the X-data matrix (which contains input measured variables) into two blocks: one representing the structured variance in X correlated with Y (which contains response variable/s) and the other one representing the structured variance in X that is orthogonal and unrelated to Y. For a discriminant or classification problem, Y is a binary dummy variable matrix where each column represents the belonging of each observation to its group (class membership). Hotelling's T^2 (a multivariate generalization of t Student's distribution tests) and DModX (a coefficient calculated using model residuals that measures how well the observations are described by the model) were applied to verify the presence of outliers and to evaluate whether a submitted sample fell within the model's Applicability Domain. The significance of the OPLS-DA model was estimated by performing Permutation tests (400 permutations) to prevent overfitting of the models. In order to increase the sample size and therefore the models' robustness, the two batches for each type of flour at each hydration level were considered separately. Class discriminating variables were analysed through one-way analysis of variance (ANOVA) followed by Duncan's *post-hoc* test at 0.05 significance level (SPSS Statistical Software, Version 25.0, IBM SPSS Inc., Armonk, New York, USA) in order to investigate significant differences between sample classes.

3. Results and discussion

3.1 Overall description of molecular properties of flour-water systems

Relaxation times and relative abundances of ^1H FID and ^1H T_2 populations of flour-water systems at each moisture content are reported in Table S2.

For all the systems analysed, the FID curves fitting displayed the existence of two proton populations, namely population A (PopA, the less mobile one) and population B (PopB, the more mobile one), relaxing in the ranges 0.0134–0.065 ms and 0.35–0.45 ms, respectively. The relative abundance of population A + population B gives 100% of the FID proton signal, although PopA was identified as the dominant FID population, encompassing 69.54–81.33% of the total observable protons. Instead, PopB represented 18.67–30.46% of the total protons.

In all the samples, the ^1H T_2 distributions of the relaxation times showed the presence of four proton populations (Fig.1), named population C (PopC), population D (PopD), population E (PopE) and population

F (PopF), relaxing in the range 0.27–1.74 ms, 3.30–7.02 ms, 11.82–27.31 ms and 43.30–85.33 ms, respectively. In all cases, PopD, PopE and PopF were not completely resolved, although sorghum flour-based doughs presented a better resolution of protons populations peaks than W, suggesting a less inhomogeneous protons exchange and molecular structure of the former.

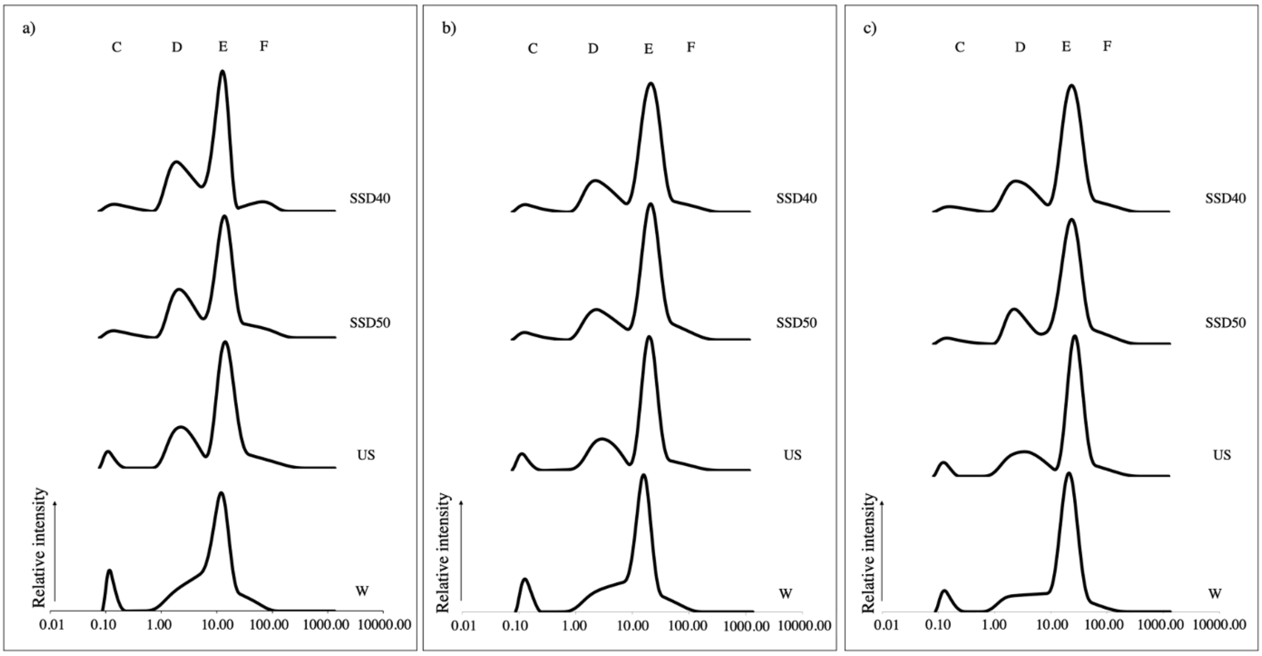

Fig. 1. Representative ^{1}H T_2 quasi-continuous distributions of flour-water systems at a) 43%, b) 48%, c) 53% moisture content.

Considering the relative abundances, populations C, D, E and F gave 100% of the ^{1}H T_2 distributions. In the ^{1}H T_2 time frame window, the dominant population was PopE, representing 48.29–60.54% of the total detectable protons, followed by PopD (16.55–29.56%), PopC (6.41–17.75%) and PopF (6.62–11.81%).

Overall, T_2 populations of W flour doughs were found consistent in terms of average relaxation times and relative abundances of different populations with previous ^{1}H NMR relaxations studies on dough (Bosmans et al., 2012; Parenti et al., 2020). According to the aforementioned authors, PopA (^{1}HFID) contains rigid CH protons of *solid-like* components of the flour, including crystalline and densely packed amorphous starch or proteins not in contact with water and protons of water molecules strongly associated with solid components. With regard to CPMG proton distribution, PopC was assigned to the more mobile CH protons of amorphous starch and gluten in little contact with confined water. PopD contains OH protons of intragranular water and starch and CH protons of gluten and exchanging protons of confined water and gluten. The most abundant proton population, PopE, was assigned to mobile OH protons of water in the extra-granular space in exchange with starch OH protons on the granule surface, and to water protons surrounding the sheets in exchange with gluten protons. The most mobile proton population, PopF, contains weakly bound protons of water, in agreement with the attributions by Wang, Ye, Li, Wei, Chen, & Zhao (2017) and Parenti et al., (2020) who characterized water mobilities in fibre-enriched and wholewheat flour doughs. Furthermore, the milling by-products (bran and germ) contained in the wholewheat flour used in our study to obtain W dough, may have affected the ^{1}H NMR profile, especially with regard to CPMG proton populations C and E, as previously stated by Hemdane et al., (2017) and Parenti et al., (2020).

Given that the proton distribution patterns of the sorghum flour-based systems were similar as those of W doughs, and taking into consideration the population assignments made in previous ^{1}H NMR relaxation studies on gluten-free cereal doughs (Hager et al., 2014), the populations assignment to different ^{1}H domains in sorghum flour-water systems could be performed in a similar way as for W.

PopA may be therefore attributed to rigid CH protons of densely packed (amorphous and crystalline) starch and of protein not in contact with water, while PopB may be assigned to more mobile protons of amorphous starch in strong interaction with water. CPMG PopC may contains CH protons of amorphous starch and protein in little contact with water, and dietary fibre constituents (i.e., arabinoxylan), thus PopD may contain OH protons of intragranular water and starch, CH protons of protein and exchanging protons of confined water and protein. PopE may contain exchanging protons of bran- and flour- related biopolymers and water interacting with these biopolymers. Finally, the most mobile population PopF may be related to the mobile or weakly bound OH protons of water.

3.2 Overall description of viscoelastic properties of flour-water systems

Dynamic rheological analysis is a powerful way to study flour dough structure and its viscoelastic properties due to its high sensitivity response to variations in structure of samples and their constituents (Song & Zheng, 2007). E', E'' and tanδ curves versus frequency for different flour-water systems at 43, 48 and 53% moisture content are presented in Figure 2. The frequency range chosen for the analysis (0.1-10 Hz) was the result of preliminary tests which had shown the greater structural fragility of sorghum systems compared to W; all the systems showed structural failure that did not allow to measure them at oscillation frequencies greater than 10 Hz. In the frequency range explored, for all the flours analysed and at each hydration level, the storage modulus (E', Figs. 2a, 2d and 2g) was higher in magnitude than the corresponding loss modulus (E'', Figs. 2b, 2e and 2h), and tanδ was in any cases < 1 (Figs. 2c, 2f and 2i) indicating solid-like properties in all samples.

W exhibited a much higher magnitude of E' (Figs. 2a, 2d and 2g) and E'' (Figs. 2b, 2e and 2f) in comparison to sorghum flour-based systems at all the moisture contents tested and at all frequencies, indicating greater viscoelasticity. Additionally, the tanδ of W samples was always the lowest among the samples analysed (Figs. 2c, 2f and 2i), which indicated the highest technological quality of W flour compared to sorghum ones, as expected (Song & Zheng, 2007).

The viscoelastic properties of sorghum flour-based systems remained substantially unchanged until a frequency of ~8 Hz, but afterwards a decrease in E' and E'' with increasing frequency was observed, indicating a weakening of the system structure with increasing mechanical stress. The fall in E' observed for all sorghum flour-water system is an indication of their poor elastic-like behaviour.

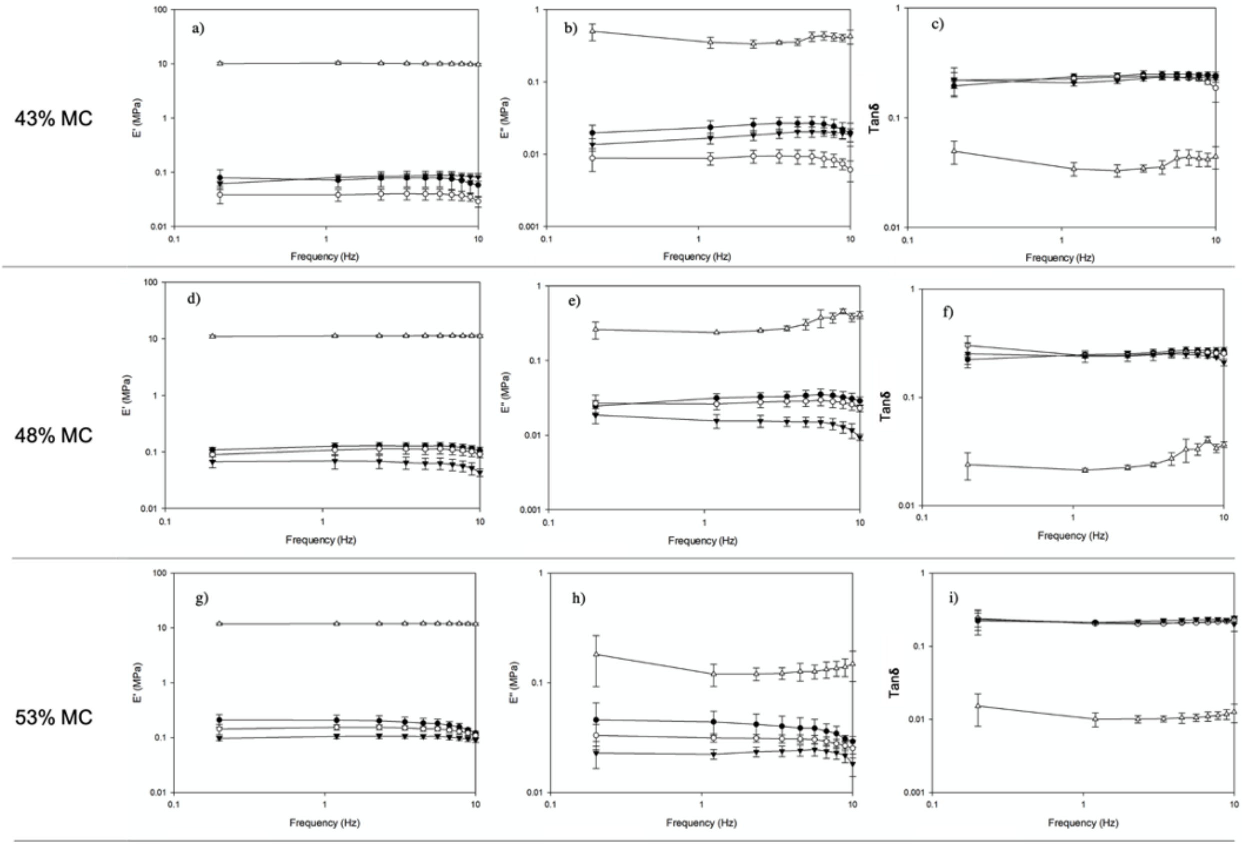

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Fig. 2. Frequency sweep parameters of flour-water systems obtained with STD (△), S (▼), SSD50 (○) and SSD40 (●) flours at 43, 48 and 53% moisture content (MC). Storage modulus (E', Figs. 2a, 2d, 2g); Loss modulus (E'', Figs. 2b, 2e, 2h); tan δ (Figs. 2c, 2f, 2i).

3.3 Multivariate outputs

In order to get deeper insights into data by providing an understanding of the main patterns as a function of sprouting, and underlying relationships between viscoelastic and molecular properties, multivariate statistics were performed. A preliminary PCA was performed to find possible outliers and to provide a general understanding of the dataset and therefore to give information of some intrinsic structures inside the data as a function of flour type and moisture content. A PCA model with three components explained 92% of the total variance (R^2), with high prediction power (Q^2 = 80%). The score plot of the first two principal components (t1/t2; Figure 3a) highlighted PC1 enabled a clear separation of doughs made from wholewheat flour (right quadrant) from those made from sorghum flours (left quadrant). The most influent variables responsible to the spatial distribution of samples (Figure 3b) for PC1 were E', E'' and $FIDT_A$, that present higher relative levels for the samples in the right quadrant and lower level for the samples in the left quadrant of Figure 3a, and T_{2C}, T_{2D} and T_{2F} that inversely present higher relative levels for the samples in the left quadrant and lower level for the samples in the right quadrant (Figure 3a). Thus, sorghum flour systems showed higher molecular and lower viscoelastic parameters than wheat ones.

PC2, on the other hand, partially matched the variation in data that contained information about the water content of the systems. In particular, from the lowest to the highest scores for PC2 (from the bottom to the top of Y axis), it was observable a samples distribution trend according to a decreasing moisture content. Specifically, all samples at 53% MC resulted located in the lower quadrants of the score plot, samples at 48% MC were located more in the centre of the plot, while samples at 43% MC were all located in the upper quadrants (Figure 3a). The most influential variables for PC2 were %PopA and %PopD, which present higher relative levels for the samples at 43% hydration and %PopB and %PopE, which present higher relative levels for the samples at 53% (Figure 3b). It has to be highlighted that this second phenomenon was a trend where little changes in the variable values from samples at 43% to samples at 53% were observed (samples at 48% hydration appeared to have average levels).

The projection of samples on the factorial space created by PC1 vs. PC3 (t1/t3; Fig. 3c) enabled to differentiate US samples at 43% and 48% MC (in the bottom quadrant) from sprouted sorghum samples (in the top and centre quadrants). Furthermore, according to PC3, it can be noticed that the S53 samples were found in the group of sprouted sorghum samples, indicating a possible similarity with them. For this third component, the predominant contribution was given by %PopC (Fig. 3d) as the variables %PopF and %PopE were not taken into account because of their coefficient's high uncertainty.

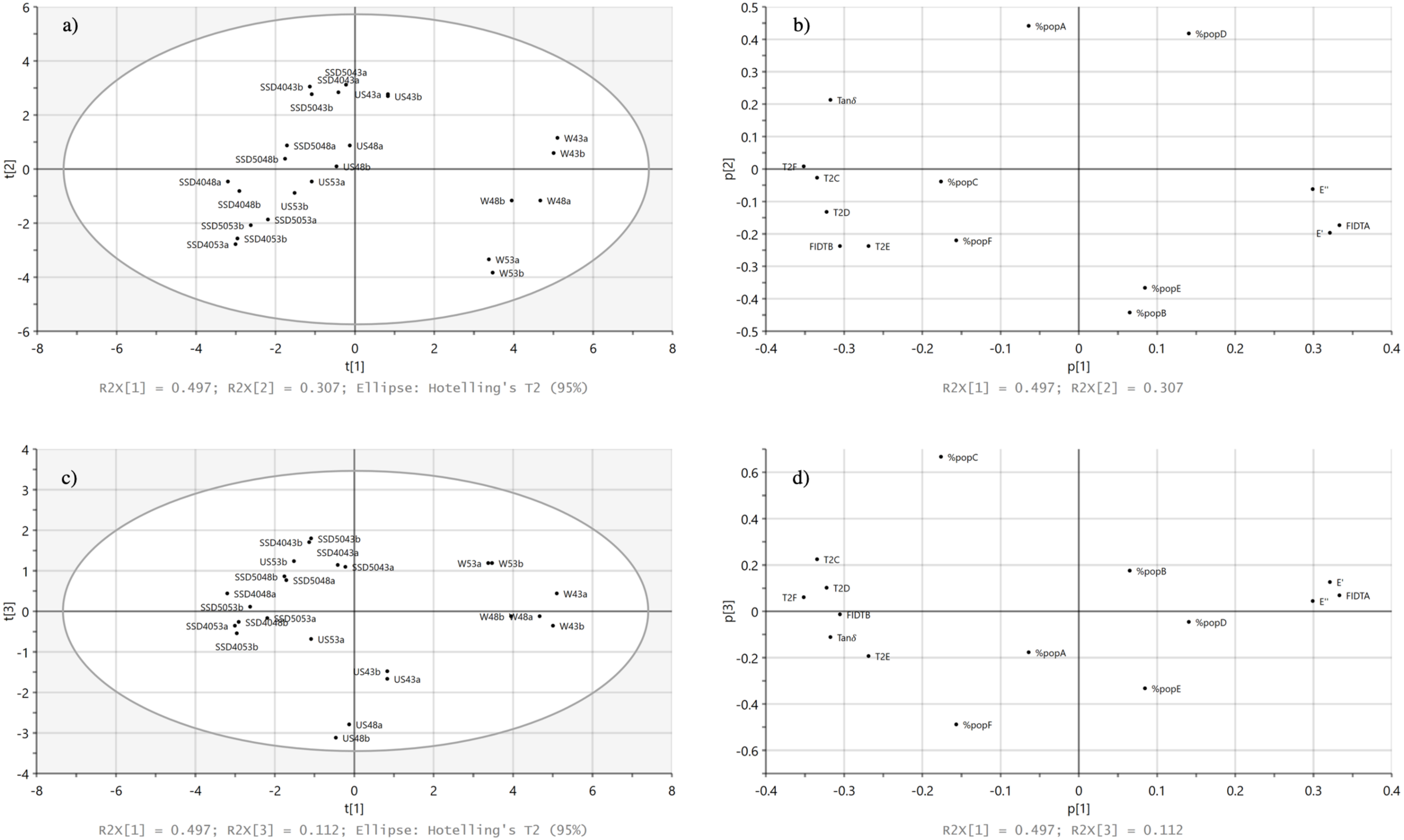

Fig. 3. Plots of the PCA model: a) score plot (t1/t2) and b) loading plot (p1/p2) of the first two principal components (PCs); c) score plot (t1/t3) and d) loading plot of the third PC versus the first PC.

An OPLS-DA model (Fig. 4) was then generated to discriminate between the samples' classes of membership, i.e., to verify whether the indications emerged from the PCA were further strengthened and whether it was possible to find further latent differences between SS and US doughs samples subclasses through of a supervised approach.

The trained OPLS-DA model showed 2 predictive and 2 orthogonal components, with an R^2 = 95% and Q^2 = 86%, indicating that the model created was suitable for discrimination of samples within classes defined on the basis of the flour used. Moreover, on the basis of Hotelling's T^2 and DModX tests (data not shown), no outliers were found, and all sample fell within the model's Applicability Domain.

The score plot (Fig. 4a) showed discrimination of the samples according to the designated classes of membership, i.e., flour-water systems made of unsprouted sorghum (US; M12.DA(1)), sprouted sorghum (SS; M12.DA(2)) and wheat flour (W; M12.DA(3)), while no discrimination was found between sprouted samples undergone to different drying treatments. This phenomenon was expected, since the information about the different drying treatments is collected in the orthogonal components (data not shown). The variables $FIDT_A$, E' and E'' (left quadrant) presented higher relative values for wheat flour samples (M12.DA(3)) confirming the considerations made with PCA. T_{2C}, T_{2D} and T_{2E} (right quadrant) presented higher relative values for sprouted sorghum flour samples (M12.DA(2)). According to the variable spatial arrangement the first group of variables presented lower relative values for sprouted sorghum flour samples and the second group lower values for wheat flour samples. Furthermore, only the %PopC variable seemed to be influent for unsprouted samples M12.DA(1) class, albeit weakly anti-correlated. Response permutation testing (400 permutations) related to M12.DA(1) revealed the reproducibility and accuracy of the original OPLS-DA model (Fig. S1).

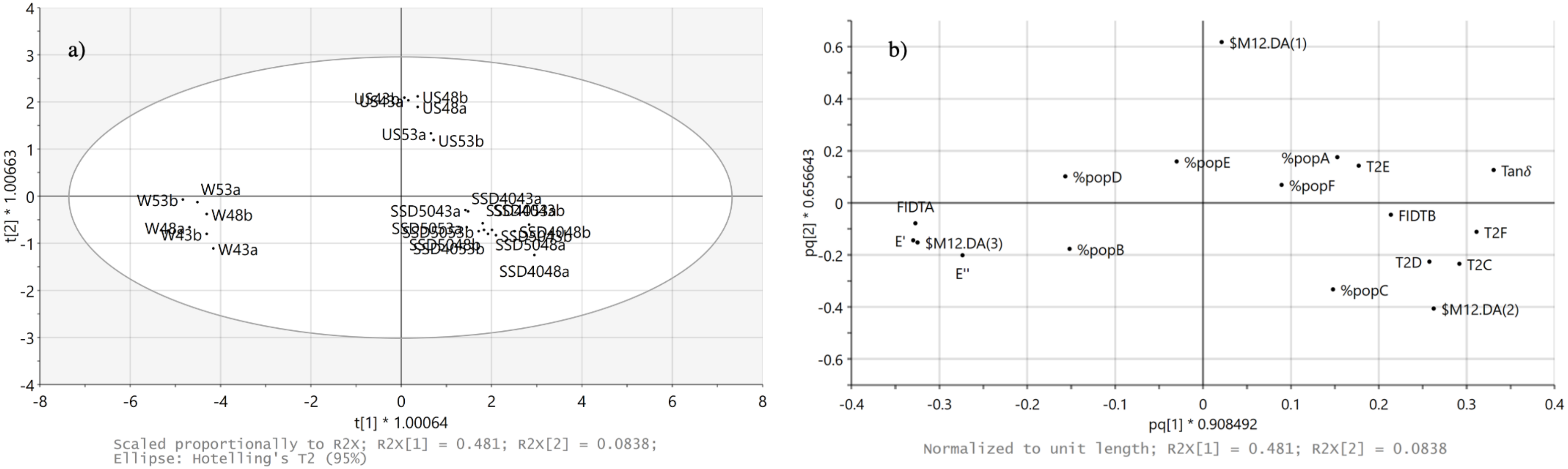

Fig. 4. OPLS-DA score plot (a) and loading plot (b) of the model discriminating between US (M12.DA(1)), SS (M12.DA(2)) and W (M12.DA(3)) flour type classes.

4. Discussion

Based on OPLS-DA output, a one-way ANOVA analysis of variance for significative variables was carried out (Table 1), in order to discuss the differences among flour-type classes. T_A from FID signal changed significantly depending on the type of flour. Wheat-based doughs showed higher relaxation time than sorghum-based systems, which means that for the latter, stronger interactions in the CH protons crystalline and amorphous starch and protein domain existed than in the former. Since starch is the main component of cereal doughs, the changes in population A may mainly reflect possible differences in wheat and sorghum starch structures, e.g., amylose and amylopectin contents, molecular weight and structural organization, amylopectin crystallinity. Moreover, higher molecular rigidity of sorghum starch and proteins domains (population A) may be attributed to the peculiar sorghum microstructure characterized by strong protein-protein and starch-protein interactions, if compared with wheat (De Mesa-Stonestreet, Alavi, & Bean, 2010; Zhu, 2014). Additionally, sorghum starch swelling power at a temperature lower than 75 °C was reported to be lower than wheat starch (Chanapamokkhot, & Thongngam, 2007), suggesting a less accessible molecular starch "environment" in sorghum, due to the hydrophobic properties of the kafirin proteins, that might have resulted in limited molecular mobility in population A.

Sprouting treatments further decreased the mobility of this population (Table 1) indicating an increase in the rigidity compared to unsprouted systems. In our previous work on the same flours, swelling power was found to decrease as a consequence of the sprouting treatment (Marchini et al., 2021), in agreement with previous studies on flours from sprouted sorghum (Chinma et al., 2015; Singh et al., 2017). The swelling power decrease upon sprouting is due to changes in starch structure mainly caused by the increase in amylose/amylopectin ratio (Marchini et al., 2021), which is a consequence of enzymatic activities triggered during sprouting that preferentially hydrolyse the amylopectin chains (Kano, Kunitake, Karakawa, Taniguchi, & Nakamuara, 1981; Marchini et al., 2021). Indeed, during sprouting, the outermost branches of amylopectin are degraded by amylolytic enzymes and are thus no longer be able to form large amylopectin crystals (Marchini et al., 2021). Losing most of their water binding ability, these smaller crystallites cannot form a three-dimensional network, resulting in a reduction of swelling power and a worsening of the pasting properties of starch when heated, and therefore in an increase in their rigidity (Li, & Yeh, 2001; Marchini et al., 2021). Since amylopectin is the primary component responsible for the swelling power of starch and amylopectin's branch chains are principally responsible for the formation of crystalline structure in starch granules (Li, & Yeh, 2001; Ai, & Jane, 2018), it could be presumable to speculate that the increase of rigidity of the population A in sprouted samples could be related to the possible higher proportion of more rigid short-chain molecules and smaller crystallites of amylopectin.

Considering the relaxation times of the three ^1H T_2 populations C, D and F, they revealed lower molecular mobility in wheat flour doughs than sorghum-based flour-water systems (significantly shorter relaxation times in W if compared to US and SS). A lower mobility indicates that the water-starch, water-gluten interactions which occurred in W doughs were stronger than those developed between water and starch, protein and bran in sorghum systems. Moreover, water-bran components (e.g., arabinoxylans) may also have had an effect on the different mobility found between wheat and sorghum flour-samples.

Previous unpublished ^{1}H NMR relaxation studies revealed that the dynamics of kafirin change remarkably little with hydration, and that most of the protein remains rigid in the presence of water (Taylor & Belton, 2002) indicating a poor plasticizing effect of water and thus the generally rather weak interaction of kafirins with water (Taylor & Belton, 2002). As discussed above, the microstructure of sorghum, characterized by high degrees of polymerization, extensive disulfide bridges and high hydrophobicity of kafirin storage proteins encapsulated in protein bodies, as well as strong protein-starch interactions, make the accessibility of proteins and starch domains to water hindered, allowing the establishment of water-solids interactions of weaker nature than those established in wheat-based samples.

A significant effect of sprouting on proton populations was also noticed on CPMG timeframe window (Table 1). Indeed, sprouting of sorghum caused an increase in the systems' mobility with respect to unsprouted flour-based systems, probably due to the hydrolytic starch breakdown caused by α-amylases during sprouting, which caused a reduction in swelling power (Marchini et al., 2021) and therefore in the ability to interact with water, as previously discussed. Moreover, the effect of sprouting on population C not only manifested in relation to its relaxation time, but also in relation to its relative abundance. Indeed, a significant increase in the relative protons abundance was observed as a result of the sprouting process. An improved protein capacity to retain water upon sprouting of these samples was previously found (Marchini et al., 2021) and generally confirmed by previous works (Singh et al., 2015; Lemmens et al., 2019; Elkhalifa, & Bernhardt, 2010). The changes in hydration properties may be related to the increased polar groups in proteins as a consequence of the enzymatic activities triggered during sprouting. The exposure of part of the water binding site on the side chain groups of protein seemingly leads to an increase in sites interacting with water (Singh et al., 2015).

Thus, remembering that PopC may contains CH protons of amorphous starch and proteins in little contact with water, and dietary fibre constituents (i.e., arabinoxylan), the higher population C proton abundance observed in SS samples compared to US ones, may be the result of the higher amount of water molecules retained by protein domain at molecular level.

Summing up, proton molecular mobility and dynamics of sprouted sorghum flour-water systems compared to unsprouted ones disclosed: (i) higher rigidity of starch domain, possibly caused by an increase of crystallinity as a consequence of sprouting; (ii) a weakening of water-starch interactions possibly due to the reduction of swelling power as an effect of sprouting; (iii) an increase of proteins-water interactions, possibly related to the enzymatic activities on proteins domain triggered during sprouting.

As far as the rheological properties are concerned, the rheological variables E' and E" were found significative in discriminating only wheat flour-based doughs from sorghum ones, while no significant changes related to sprouting and/or post-sprouting drying treatments were detected. W doughs proved to be the samples with the greatest viscoelastic properties, as expected. The poor viscoelasticity of sorghum-based systems may be related to the lack of gluten network and to the high hydrophobicity degree of kafirins. Indeed, the poor viscoelastic properties of sorghum kafirins had been previously observed by Oom, Pettersson, Taylor, & Stading (2008) by performing dynamic measurements in shear on kafirin resins by means of a rheometer. Furthermore, none of the kafirin-starch dough-like systems investigated by the same authors developed into a viscoelastic dough, due to kafirins' high hydrophobicity. Overall, the poor viscoelastic properties (E' and E") observed for sorghum flour-based systems were related to their more mobile molecular systems, as underlined by the multivariate statistics. Interestingly, the significant changes in molecular dynamics observed in SS samples due to sprouting did not affect viscoelastic properties of same samples indicating, in other words, a non-worsening of the rheological properties of sorghum systems as a function of sprouting. This can be of relevance in applications where the brief is the increase of the nutritional value of food and a not high viscoelastic performance is required. An improvement of the nutritional value of food products, such as unleavened/flat bread, by the use of sprouted sorghum flour, could be of great relevance in those countries where food security is not a status but a goal to be achieved, sprouting is traditionally empirically performed, sorghum is a staple food, flat bread preparation is part of the gastronomic culture, id est in most of African countries.

Since the PCA showed a trend contingent on the moisture content of the samples (PC2, Section 3.3 and Figs. 3a and 3b), the effect of the water content on PC2 most influencing variables was further investigated by performing a one-way ANOVA. Table 2 shows the comparison as a function of the hydration level (43%, 48% and 53%), independently of the type of flour. All analysed proton populations proved to be sensitive to water content variations: a significative ($p \leq 0.05$) reduction in %PopA (and a consequent increase in %PopB) and %PopD was observed with the increase in moisture content, while an increase in the number of protons of the more mobile population (%PopE) was recorded. These changes in the proton populations abundances as a function of water content agreed with previous findings (Assifaoui et al., 2006; Doona, & Baik, 2007) and indicated an increase in molecular mobility due to the higher water available in the system. Besides, changes in moisture content did not significantly affect the viscoelastic properties of the doughs at this dimensional scale of investigation.

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Table 1. One-way ANOVA of significative variables defined by OPLS-DA performed on dough samples grouped according to OPLS-DA output.

	NMR					DMA	
sample	T_A	T_{2C}	%PopC	T_{2D}	T_{2F}	E'	E"
US	0.0139 ± 0.0001b	0.7636 ± 0.3102b	10.32 ± 4.58b	4.3076 ± 0.5359b	63.5595 ± 3.8293b	0.09 ± 0.02b	0.02 ± 0.01b
SS	0.0136 ± 0.0002c	1.5995 ± 0.2068a	15.23 ± 2.08a	6.1884 ± 1.1316a	78.0115 ± 6.6621a	0.12 ± 0.05b	0.03 ± 0.01b
W	0.0164 ± 0.0001a	0.3285 ± 0.0618c	11.28 ± 2.12b	3.459 ± 0.2202c	46.0624 ± 4.1851c	10.99 ± 1.01a	0.31 ± 0.15a

Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$). US, unsprouted sorghum flour; SS, flours from sprouted sorghum; W, organic wholewheat flour.

Table 2. One-way ANOVA of most important variables for the second principal component PC2 of PCA model, performed on dough samples grouped according to moisture content (MC).

	NMR				DMA
MC (%)	%PopA	%PopB	%PopD	%PopE	tanδ
43	79.76 ± 1.61a	20.24 ± 1.61c	27.78 ± 1.91a	52.62 ± 2.73c	0.17 ± 0.10
48	77.08 ± 1.67b	22.92 ± 1.67b	22.07 ± 1.84b	56.49 ± 2.26b	0.21 ± 0.10
53	73.37 ± 2.64c	26.63 ± 2.64a	18.26 ± 1.87c	57.69 ± 2.75a	0.16 0.09

Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$).

5. Conclusion

The effect of sprouting on the technological functionalities of sorghum flour-water systems by means of LR ^{1}H NMR and Dynamic Mechanical Analysis (DMA) was assessed. The ^{1}H T_2 results showed greater molecular mobility in the sorghum-based systems compared to wheat-based ones, that could explain their poorer viscoelastic properties. In addition, sprouting increased the molecular mobility of the samples. However, these changes were not reflected on the flour-water system viscoelastic properties as they did not change upon sprouting.

Overall, as the use of sorghum flours find application where high viscoelastic performance and/or intensive starch gelatinization is not demanded, it could prove useful to study the use of these flours in a blend with other raw materials, in order to develop finished products with high nutritional profile and satisfactory technological and organoleptic properties. Even more importantly, and especially for those countries where sorghum is a staple of the diet, the use of sprouted sorghum flour should be encouraged rather than the unsprouted one in the applications mentioned above. The former is characterized by a significantly higher nutritional profile than the latter, while both produce flour-water systems with comparable viscoelastic properties, as demonstrated in this work.

Supplementary materials

Table S2. Proximate composition of flour samples. Carbohydrates, protein, fat and ash are expressed as % (g/100 g) on dry basis, while moisture content is expressed as % (g/100 g) on wet basis (w.b.).

	Proximate composition				
Sample	Carbohydrates	Protein	Fat	Moisture	Ash
US	85.3 ± 0.1	11.3 ± 0.3	3.7 ± 0.0	11.3 ± 0.2	1.4 ± 0.0
SSD50	84.5 ± 0.5	11.0 ± 0.5	3.3 ± 0.0	12.7 ± 0.0	1.3 ± 0.0
SSD40	83.6 ± 0.1	12.0 ± 0.0	3.1 ± 0.0	12.7 ± 0.0	1.3 ± 0.0
W	81.8 ± 0.2	14.8 ± 0.3	1.8 ± 0.0	10.7 ± 0.0	1.5 ± 0.0

US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; W, organic wholewheat flour.

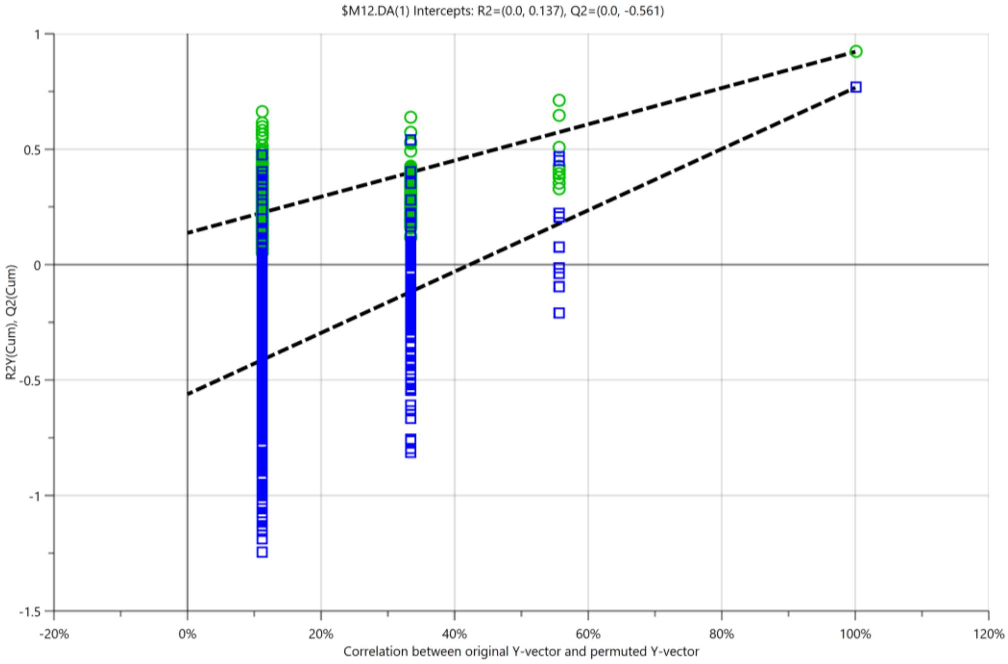

Fig. S1. Validate plot of the two-dimensional OPLS-DA model referred to M12.DA(1) sample class. The plot is constructed by letting the Y-axis represent the R^2Y (○) and Q^2Y (■) of the model and by assigning the X-axis to the correlation between permuted and original Y-vector. R^2Y (0.1) and Q^2Y (-0.6) intercepts are substantially lower than the original OPLS-DA model's values; similarly, Validate Models for M12.DA(2) and M12.DA(3) estimated both "permuted" R^2Y-values = 0.1, and Q^2Y-values = -0.5 for M12.DA(2) and Q^2Y-values = -0.6 for M12.DA(3) respectively (data not shown), revealing the reproducibility and accuracy of the original OPLS-DA model.

Table S2. Relaxation times (T_A, T_B, T_{2C}, T_{2D}, T_{2E} and T_{2F}) and relative abundances (%PopA, %PopB, %PopC, %PopD, %PopE and %PopF) of ^{1}H FID and T_2 proton populations of flour-water systems at three different moisture content (MC; 43, 48 and 53%, g water/100 g sample).

		^{1}H FID				^{1}H T_2							
		PopA		PopB		PopC		PopD		PopE		PopF	
	MC (%)	T_A (ms)	(%)	T_B (ms)	(%)	T_{2C} (ms)	(%)	T_{2D} (ms)	(%)	T_{2E} (ms)	(%)	T_{2F} (ms)	(%)
US	43	0.0139 ± 0.0001bB	81.33 ± 0.24aA	0.38 ± 0.00cC	18.67 ± 0.24cC	0.50 ± 0.08cB	7.94 ± 0.76bD	3.84 ± 0.16cB	29.56 ± 0.39aA	18.67 ± 0.43cA	55.21 ± 0.38bA	60.09 ± 2.76bB	7.29 ± 0.52cB
	48	0.0139 ± 0.0001bB	78.76 ± 1.02bA	0.40 ± 0.01bB	21.24 ± 1.02bD	0.66 ± 0.11bC	6.41 ± 0.46cC	4.18 ± 0.18bC	24.40 ± 0.53bA	22.46 ± 1.25bB	57.37 ± 1.01aB	65.63 ± 2.68aC	11.81 ± 1.35bA
	53	0.0141 ± 0.0001aB	75.89 ± 1.94cA	0.41 ± 0.01aC	24.11 ± 1.94aC	1.15 ± 0.18aB	16.22 ± 2.53aA	4.93 ± 0.37aB	20.51 ± 2.29cA	27.31 ± 1.79aA	54.9 ± 3.58bC	65.68 ± 2.77aC	8.36 ± 1.18aC
SSD50	43	0.0136 ± 0.0001bC	80.29 ± 0.57aB	0.38 ± 0.00cB	19.71 ± 0.57cB	1.42 ± 0.16cA	17.75 ± 2.43aA	5.07 ± 0.67cA	27.34 ± 1.05aB	16.16 ± 1.12cC	48.29 ± 1.66cD	71.59 ± 5.15bA	6.62 ± 0.93cB
	48	0.0136 ± 0.0002bC	77.92 ± 0.81bB	0.40 ± 0.01bB	22.08 ± 0.81bC	1.54 ± 0.15bB	15.72 ± 1.68bA	5.89 ± 0.69bB	21.95 ± 0.91bC	19.7 ± 0.85bC	54.93 ± 1.27bC	78.37 ± 5.43aB	7.40 ± 0.66bC
	53	0.0138 ± 0.0001aC	74.27 ± 0.50cB	0.43 ± 0.01aB	25.73 ± 0.50aB	1.69 ± 0.16aA	14.18 ± 1.49cB	6.68 ± 0.88aA	17.98 ± 0.62cB	24.46 ± 1.26aB	57.35 ± 0.94aB	75.90 ± 4.38aB	10.50 ± 1.04aB
SSD40	43	0.0135 ± 0.0001bD	80.21 ± 0.44aB	0.40 ± 0.00cA	19.79 ± 0.44cB	1.40 ± 0.14bA	14.92 ± 1.76abB	5.10 ± 0.54bA	25.50 ± 0.88aC	17.61 ± 0.54cB	51.83 ± 1.13cC	73.34 ± 2.80cA	7.75 ± 0.48cAB
	48	0.0134 ± 0.0001cD	76.87 ± 0.44bC	0.43 ± 0.00bA	23.13 ± 0.44bB	1.72 ± 0.18aA	15.44 ± 2.07aA	7.02 ± 1.26aA	19.49 ± 1.25bD	23.55 ± 1.10bA	54.87 ± 1.92bC	85.33 ± 6.72aA	10.19 ± 1.34bB
	53	0.0136 ± 0.0001aD	73.78 ± 0.67cB	0.45 ± 0.01aA	26.22 ± 0.67aB	1.74 ± 0.17aA	13.88 ± 0.81bB	6.90 ± 0.77aA	16.55 ± 0.65cC	26.69 ± 1.20aA	57.85 ± 0.57aB	81.07 ± 4.44bA	11.72 ± 0.93aA
W	43	0.0164 ± 0.0001bA	77.18 ± 0.40aC	0.35 ± 0.00cD	22.82 ± 0.40cA	0.27 ± 0.03cC	9.79 ± 0.28cC	3.30 ± 0.16bC	28.19 ± 2.12aB	11.82 ± 0.30cD	53.82 ± 0.85cB	43.30 ± 4.88cC	8.21 ± 1.27aA
	48	0.0164 ± 0.0002bA	74.75 ± 0.47bD	0.37 ± 0.00bC	25.25 ± 0.47bA	0.34 ± 0.04bD	10.27 ± 0.88bB	3.47 ± 0.18bD	22.62 ± 0.59bB	14.81 ± 0.44bD	59.15 ± 0.71bA	45.83 ± 1.30bD	7.96 ± 0.30aC
	53	0.0165 ± 0.0002aA	69.54 ± 1.15cC	0.40 ± 0.01aD	30.46 ± 1.15aA	0.38 ± 0.06aC	14.25 ± 1.16aB	3.63 ± 0.21aC	18.26 ± 0.57cB	18.68 ± 0.40aC	60.54 ± 0.94aA	49.49 ± 3.34aD	6.94 ± 0.27bD

Values are expressed as mean ± standard deviation (n = 24). Different letters indicate significant difference among samples (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$), where different lowercase letters indicate significant differences between samples at different MC made of the same type of flour, while different capital letters indicate significant differences between samples made by different flour types at the same MC. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; W, organic wholewheat flour.

Table S3. Storage modulus (E'), loss modulus (E") and Tanδ of flour-water systems at three different moisture content (MC, 43, 48 and 53%) recorded at a single frequency of 5.6 Hz.

	MC (%)	E" (MPa)	E" (MPa)	Tanδ
US	43	0.09 ± 0.01bB	0.02 ± 0.00aB	0.23 ± 0.01aA
	48	0.06 ± 0.01cB	0.01 ± 0.00bB	0.25 ± 0.02aB
	53	0.11 ± 0.01aB	0.02 ± 0.00aB	0.23 ± 0.02aA
SSD50	43	0.04 ± 0.01cB	0.01 ± 0.00bB	0.23 ± 0.01bA
	48	0.11 ± 0.02bB	0.03 ± 0.00aB	0.26 ± 0.01aAB
	53	0.14 ± 0.01aB	0.03 ± 0.00aB	0.21 ± 0.00cB
SSD40	43	0.08 ± 0.03cB	0.03 ± 0.01bB	0.25 ± 0.01aA
	48	0.13 ± 0.02bB	0.04 ± 0.01aB	0.27 ± 0.02aA
	53	0.18 ± 0.04aB	0.04 ± 0.01aB	0.21 ± 0.00bB
W	43	10.01 ± 0.47cA	0.42 ± 0.06aA	0.04 ± 0.01aB
	48	11.28 ± 0.32bA	0.38 ± 0.10aA	0.03 ± 0.01bC
	53	11.99 ± 0.48aA	0.13 ± 0.02bA	0.01 ± 0.00cC

Values are expressed as mean ± standard deviation ($n \geq 5$). Different letters indicate significant difference among samples (one-way ANOVA with Duncan's post-hoc test, $p \leq 0.05$), where different lowercase letters indicate significant differences between samples at different MC made of the same type of flour, while different capital letters indicate significant differences between samples made by different flour types at the same MC. US, unsprouted sorghum flour; SSD50, flour from sprouted sorghum dried at 50 °C for 6 h; SSD40, flour from sprouted sorghum dried at 40 °C for 12 h; W, organic wholewheat flour.

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Chapter 4

Functional properties of sustainable composite flours from sorghum, tapioca and cowpea

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Author contribution: M.M.: data acquisition, elaboration and discussion, conceptualization, manuscript writing.

Abstract

Using composite flours by accessible raw materials for food production may be of great interest for developing countries, cutting costs of importing wheat flour, bolstering domestic agricultural production, and increasing protein and biocomponent provision. This work evaluated the technological functionality of sustainable composite flours made from tapioca, sprouted sorghum, cowpea, and wheat flours [in relative abundance to 50%, 33% and 25% (w/w) flour basis]. PCA output revealed that when used together to make a 50% w/w blend, sprouted sorghum and tapioca exhibited technological properties similar to wheat making this blend particularly interesting when used in the production of bakery products which do not require the viscoelastic properties of gluten. Since cowpea flour is recommended to enhance nutrients, a flour of sprouted sorghum, tapioca and cowpea is ideal and also sustainable, since its raw materials are sourced locally. Furthermore, PCA showed that the composite flour made up of sprouted sorghum flour, tapioca, cowpea and wheat flour at 25% w/w offers a good compromise between technological and nutritional qualities, while reducing imports of wheat flour and cassava post-harvest losses. These results promise technologically satisfactory, highly nutritional, sustainable bakery products.

Keywords

Germination, sorghum, tapioca, cowpea, composite flours, physicochemical properties, thermal properties, pasting properties

Abbreviations

SS, sprouted sorghum flour; T: tapioca; W: wheat flour; C: cowpea flour; alveographic parameters - W (J 10^{-4}), baking strength; P/L ratio, curve configuration ratio; P (mm), dough tenacity; L (mm), dough extensibility; SS_T: sprouted sorghum and tapioca flour blend; SS_W: sprouted sorghum and wheat flour blend; SS_C: sprouted sorghum and cowpea flour blend; SS_W_T: sprouted sorghum, wheat and tapioca flour blend; SS_C_W: sprouted sorghum, cowpea and wheat flour blend; SS_C_T: sprouted sorghum, cowpea and tapioca flour blend; SS_C_W_T: sprouted sorghum, cowpea, wheat and tapioca flour blend; d.b., dry basis; f.b., flour basis; WHC, water holding capacity; OHC, oil holding capacity; R/T, room temperature; S_p, Swelling Power; DSC, differential scanning calorimetry; ΔH, enthalpy; T_{on}, onset temperature; T_p, peak temperature; T_{off}, offset temperature; MVAG, Micro-Visco Amylograph; BD, breakdown; SB, setback; SD, standard deviation; ANOVA, analysis of variance; PCA, principal component analysis; PC, principal component; ALCs, amylose-lipid complexes.

1. Introduction

Fighting hunger and reducing the expenditure on imported foods are a priority for most low- to middle-income developing countries. Scientific and technological innovations supported by public investment and suitable policies should aim at promoting the use of country-specific and locally available food crops, as well as encouraging agri-food development, reducing import costs, and providing much-needed income for smallholders (Abass et al., 2016). In view of this, the use of composite flours for food production has been proposed as an advantageous approach for developing countries, encouraging the use of domestic agricultural products and, at the same time, promoting a better supply of proteins and biocomponents for human nutrition (Hugo, Rooney, & Taylor, 2000; Bugusu, Campanella, & Hamaker, 2001; Noorfarahzilah, Lee, Sharifudin, Fadzelly, & Hasmadi, 2014).

Sorghum (*Sorghum bicolour* [L.] Moench) represents a dietary staple for more than 500 million poor and food-insecure populations in the developing world, especially in Africa, and it is therefore potentially suitable for being used in composite flours (Hugo et al., 2000; Kulamarva, Sosle, & Vijaya Raghavan, 2009; Xu, 2019). Sprouting of sorghum - along with post-sprouting drying treatments - have been documented as sustainable process to improve the nutritional profile as well as to change the functional properties of the derived flour. Specifically, sprouting of sorghum leads to an increase in the amount of bioactive compounds and their *in vitro* bioavailability, and antioxidant activity, as well as to an increase in flour's solubility, water and oil holding capacity, foam and emulsion capacity and stability and to a worsening in its starch pasting properties (Abd Elmoneim & Bernhardt, 2010; Afify, El-Beltagi, Abd El-Salam, & Omran, 2011; Marengo, Bonomi, Marti, Pagani, Abd Elmoneim, & Iametti, 2015; Singh, Rehal, Kaur, & Jyot, 2015; Singh, Sharma, & Singh, 2017; Lemmens et al., 2019; Section B, Chapter 1 and Chapter 2).

In this perspective, the use of sprouted sorghum in combination with other staples, such as legumes and tapioca, could be useful to develop sustainable bread with satisfactory technological and sensory properties as well as an improved nutritional profile.

Cassava (*Manihot esculenta* Crantz) is a starchy root crop common among the lowland, sub-humid tropics of Africa, and a primary source of calories for around two-fifths of all Africans (Zhu, 2015). Its high tolerability to drought and harsh climatic conditions, a high yield in poor soil and its around-the-year availability, make cassava a dependable crop for food security and various traditional food applications (Zhu, 2015). In addition, its flour (tapioca) is considered suitable for different food productions as exhibiting excellent starch physicochemical and pasting properties together with a mild non-cereal taste. Therefore, also cassava flour can partially replace wheat flour in bakery products, thereby reducing both expenditure on wheat imports in low-income countries and cassava post-harvest losses (Falade, & Akingbala, 2010; Abass et al., 2016).

Meanwhile, cowpea (*Vigna unguiculata* L. Walpers), also known as black eye pea, is one of the most important edible legumes contributing to food security and maintenance of the environment for millions of small-scale farmers in developing countries (Da Silva, da Costa Santos, Junior, da Silva, dos Santos, & Siviero, 2018).

Around 83% of the world's overall production of cowpea is from Africa, with over 80% of African production in West Africa (Kebede, & Bekeko, 2020). Cowpea is a good cheap source of protein and carbohydrate, as well as fibre and bioactive compounds (Jayathilake et al., 2018; Oyeyinka et al., 2020). Despite this, the use of cowpea has mainly been limited to traditional preparations (Oyeyinka et al., 2018), e.g., as a stand-alone boiled vegetable or in mixed dishes. To encourage higher consumption of this legume, recent efforts have increasingly focused on its use in composite flour to improve both the technological behaviour and nutritional profile of bakery products, revealing good functional properties of both its protein and starch components (Kerr, Ward, McWatters, & Resurreccion, 2000; Akubor, 2003; Phebean, Akinyele, Toyin, Folasade, Olabisi, & Nnenna, 2017; Ngoma, et al., 2018).

Given the above, the aim of this work was to study the functional features of sustainable composite flours with potential high nutritional value constituted by sprouted sorghum, tapioca, and cowpea flours.

2. Materials and methods

2.1 Flours

Wholemeal flour from sprouted sorghum was obtained as described in a previous work (Section B, Chapter 1). Briefly, kernels were sprouted at 25 °C for 72 h and dried at 40 °C for 12 h, milled with a lab-scale mill (Labormill, BONA, Monza, Italy) to produce refined flour, middlings, and bran, which were reconstituted to wholemeal sorghum flour (SS). Tapioca (T) and cowpea wholemeal (C) flours were purchased from Molino Bongiovanni S.r.l. (Villanova Mondovì, CN, Italy). Wholewheat flour (W) (W= 240 J 10^{-4} and P/L = 0.55) was purchased from Molino Grassi S.p.A. (Fraore, PR, Italy).

2.2 Blend preparation

Seven composite flours were formulated by blending SS with T, W and C flours in different proportions to obtain the following blends: 50:50 w/w flour basis (f.b.) (SS_T; SS_W; SS_C); 33:33:33 w/w f.b. (SS_W_T; SS_W_C; SS_C_T) and 25:25:25:25 w/w f.b. (SS_C_W_T). Flours were mixed for 15 min at medium speed using a Kitchen Aid Professional Mixer (KitchenAid, St. Joseph, Michigan, USA) equipped with a flat beater mixer, and then mixed manually for a further 5 min.

2.3 Proximate composition

The proximate composition of SS, T, C and W flours was measured by determining total protein (AACCI method 46–12.01), fat (AACCI Method 30–25.01), and ash (AACCI method 08–01.01) contents. Moisture content was measured by oven drying at 130 °C to constant weight (adapted from AACCI method 44–15.02), while the carbohydrates were determined by difference. The proximate composition of the composite flours was calculated on the basis of the chemical composition of their constituents and their percentage of addition. Protein, fat, ash and carbohydrate were expressed as g/100 g on dry basis (d.b.), moisture content as g/100 g on wet basis (w.b.).

2.4 Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

WHC and OHC were determined following the method described by Marchini et al. (2020). Briefly, 100 mg of flour was added to 1 ml of distilled water (WHC) or sunflower oil (OHC), shaken with a vortex for 30 s, and then allowed to rest at R/T for 30 min. After centrifugation (Centrifuge 5417 R, Eppendorf AG, Germany) at 2061 g (4000 rpm) for 20 min, the supernatant was decanted. WHC and OHC were calculated as the ratio between the grams of water or oil retained per gram of solid.

2.5 Swelling power (S_p)

S_p was measured following the method previously described by Marchini et al. (2020). Briefly, flour suspensions (2% w/v) were heated in a water bath at a selected temperature (60, 70, 80 or 90 °C) for 1 h and cooled at 30 °C for 30 min. After centrifugation (8243 g/8000 rpm for 20 min), the precipitated part was weighed. The S_p was calculated as the ratio between the weight of the pellet and the weight of the flour.

2.6 Thermal properties

The flours' thermal properties were determined using a Differential Scanning Calorimeter (DSC Q100 TA Instruments, USA), calibrated with indium (melting point: 156.6 °C, melting enthalpy: 28.71 J g^{-1}) and mercury (melting point: − 38.83 °C, melting enthalpy: 11.44 J g^{-1}) following the method previously described by Marchini et al. (2020). Briefly, flour-water suspensions were obtained by mixing flour and water in a ratio of 1:3 v/w and left to equilibrate overnight at R/T. An aliquot of the suspension (5-10 mg) was placed in stainless steel pans (Perkin Elmer, USA) hermetically sealed, quench cooled to 30 °C and then heated to 120 °C at 5 °C/min, using an empty pan as reference. The enthalpy (ΔH, J g^{-1}), onset (T_{on}, °C), peak (T_p), and offset (T_{off}, °C) temperatures of the observed transitions were extrapolated by heat flow curves using Universal Analysis Software, Version 4.5A (TA Instruments, USA).

2.7 Pasting properties

The pasting properties of the flours were measured by means of a Micro-Visco-Amylograph device (MVAG, Brabender GmbH & Co. KG, Duisburg, Germany) as described by Marti, Cardone, Nicolodi, Quaglia, & Pagani, (2017). Briefly, 12 g of flour was dispersed in 100 mL of a 1 mM aqueous $AgNO_3$ solution and mixed at 250 rpm. The temperature profile applied was the following: heating from 30 °C to 95 °C at a rate of 3 °C/min, holding at 95 °C for 20 min, cooling from 95 °C to 30 °C at a cooling rate of 3 °C/min, and holding at 30 °C for 1 min. The 1 mM aqueous $AgNO_3$ solution was used to inhibit α-amylase activation during analysis. The parameters calculated from the pasting curve were the following: pasting temperature (pasting T, temperature at which gelatinization begins, °C), peak viscosity (maximum viscosity value of the slurry during gelatinization, BU), peak temperature (peak T, temperature at which peak viscosity occurs, °C), final viscosity (viscosity of the slurry at the end of the test, BU), breakdown (BD, difference between peak viscosity and minimum viscosity during the holding period, BU) and setback (SB, difference between peak and final viscosities, BU).

2.8 Statistical analysis

All analyses were performed in triplicate and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test at 0.05 significance level (SPSS Statistical Software, Version 25.0, IBM SPSS Inc., USA) was performed to assess significant differences between the samples.

Data were processed with SIMCA® Software (Version 16.0.1, Umetrics Suite, Sartorius Stedim Biotech, Umeå, Sweden) using multivariate statistics to obtain a global overview of the whole dataset. Unsupervised Principal Component Analysis (PCA) was performed with mean centring and unit variance (UV) scaling as data pre-treatment. The dataset consisted of values obtained from the flours' proximate composition and functional, thermal and pasting properties. As regards the proximate composition, the dataset included the carbohydrates (carbs), protein, fat, moisture content (MC) and ash. Functional properties included WHC, OHC and S_p measured at different temperature (S_p_60, S_p_70, S_p_80, S_p_90). Thermal properties included temperatures and enthalpies of the gelatinization (T_{on}_gel, T_p_gel, T_{off}_gel, ΔH_gel) and amylose-lipids transitions ALC (T_{on}_ ALC, T_p_ ALC, T_{off}_ ALC, ΔH_ ALC). Pasting properties included pasting temperature (pasting T), peak viscosity, peak temperature (peak T), final viscosity, breakdown (BD) and setback (SB).

3. Results

The proximate composition as well as the functional, thermal, and pasting properties of SS flour were reported in the aforementioned study (Section B, Chapter 1).

3.1 Proximate composition

Among the gluten-free composite flours, SS_T showed the highest content of carbohydrates, and the lowest amount of protein, fat and ash, which reflects the chemical composition of tapioca (Table 1).

On the other hand, SS_C flour had the lowest carbohydrate content and the highest protein, fat and ash content, reflecting the proximate composition of C. As expected, the SS_C_T sample showed an intermediate composition between the two described above, and similar to the composition of W.

The addition of W acted heterogeneously on the proximate composition of the blends. Mixing W with SS at 50% w/w resulted in a flour with more protein and ash but fewer carbohydrates and fats than SS alone. Similarly, the SS_T_W blend presented more protein, fat and ash but fewer carbohydrates than SS_T, while exactly the opposite was observed when comparing SS_C_W with SS_C. Only slight differences were observed when comparing SS_C_T_W with SS_C_T.

Table 1. Proximate composition of analyzed flour blends. Carbohydrates, protein, fat and ash contents are expressed as g/100 g dry basis (d.b.), while moisture is expresses as g/100 g wet basis (w.b.).

	Carbohydrates	Protein	Fat	Moisture	Ash
SS*	83.63 ± 0.07d	11.97 ± 0.01g	3.07 ± 0.02a	12.67 ± 0.03a	1.33 ± 0.00f
T	99.28 ± 0.01a	0.58 ± 0.02l	0.04 ± 0.00m	11.35 ± 0.02cde	0.10 ± 0.01i
W	81.84 ± 0.23g	14.83 ± 0.28d	1.84 ± 0.03e	10.71 ± 0.03e	1.48 ± 0.01d
C	68.18 ± 0.11l	27.39 ± 0.01a	1.36 ± 0.00l	12.00 ± 1.00bc	3.07 ± 0.03a
SS_T	91.55 ± 0.01b	6.21 ± 0.01i	1.54 ± 0.01h	11.60 ± 0.01cd	0.70 ± 0.00h
SS_W	82.87 ± 0.05f	13.28 ± 0.07f	2.45 ± 0.02b	11.29 ± 0.00de	1.40 ± 0.00e
SS_C	75.88 ± 0.09i	19.71 ± 0.01b	2.21 ± 0.01c	12.34 ± 0.49ab	2.20 ± 0.02b
SS_T_W	88.16 ± 0.06c	9.11 ± 0.10h	1.64 ± 0.02f	11.57 ± 0.02cd	0.97 ± 0.00g
SS_C_W	77.81 ± 0.04h	18.04 ± 0.09c	2.08 ± 0.01d	11.77 ± 0.32bcd	1.96 ± 0.01c
SS_C_T	83.64 ± 0.06d	13.27 ± 0.00f	1.48 ± 0.01i	11.99 ± 0.32bc	1.49 ± 0.01d
SS_C_T_W	83.26 ± 0.02e	13.68 ± 0.07e	1.58 ± 0.01g	11.68 ± 0.23bcd	1.49 ± 0.01d

*SS data on proximate composition were presented in a previous work (Section B, Chapter 1). SS, sprouted sorghum flour; T, tapioca; W, wheat flour; C, cowpea flour; SS_T, sprouted sorghum and tapioca flour blend; SS_W, sprouted sorghum and wheat flour blend; SS_C, sprouted sorghum and cowpea flour blend; SS_W_T, sprouted sorghum, wheat and tapioca flour blend; SS_C_W, sprouted sorghum, cowpea and wheat flour blend; SS_C_T, sprouted sorghum, cowpea and tapioca flour blend; SS_C_W_T, sprouted sorghum, cowpea, wheat and tapioca flour blend. Proximate composition values are expressed as mean ± SD (n=3). Values followed by different letters in each column are significantly different ($p \leq 0.05$).

3.2 Water holding capacity (WHC), oil holding capacity (OHC) and swelling power (S_p)

Taking into consideration only wheat-free blends, SS_C showed the highest WHC, which was reasonable, since it was composed by the flours with the highest ability to hold water, followed by SS_C_T and SS_T (Table 2).

In all C-based blends, the addition of W, with a consequent decrease in C and SS percentages, reduced the flours' WHC. The same effect was observed when W was combined with SS. A worsening in WHC composite flours with the addition of W was related to the low water holding capacity recorded for this latter.

With regard to OHC, by the comparison of the wheat-free composite flours, SS_C_T showed the highest OHC, followed by SS_C and SS_T, which is to be expected in light of cowpea flour's superior OHC compared to wheat and tapioca (Table 2), confirming previous findings (Melini, Melini, Luziatelli, & Ruzzi, 2017).

Despite the lower OHC measured for W compared to SS and C, its addition to the 50:50 w/w blends and SS alone caused an increase in OHC, while the opposite effect was observed after its addition to SS_C_T.

S_p of flours and their blends are reported in Table 2. As expected, an overall increase in S_p with rising temperature was recorded for all flours (Table 2). Furthermore, almost all the blends showed higher S_p with a rise in temperature than the SS sample. Comparing the wheat-free composite flours, SS_C_T showed the highest increase in S_p with a rise in temperature, followed by SS_C and SS_T.

When considering the S_p at each temperature for the wheat-free blends, SS_T showed the highest value at 60 °C, while SS_C_T presented the highest at 70, 80 and 90 °C. An intermediate behaviour was observed for SS_C. In general, flours composed using W (SS_W, SS_T_W, SS_C_W and SS_C_T_W) did not show better

S_p values than the equivalent wheat-free blends (SS, SS_T, SS_C and SS_C_T, respectively) in the 60 – 80 °C range. Only at 90 °C the S_p of the blends increased by adding W.

Table 2. Water holding capacity, oil holding capacity and swelling power of flours and their blends.

	WHC (g/g)	OHC (g/g)	S_p (g/g)			
	25 °C	25 °C	60 °C	70 °C	80 °C	90 °C
SS*	1.68 ± 0.05d	1.05 ± 0.00de	5.39 ± 0.24bC	5.35 ± 0.23deC	6.86 ± 0.24dB	7.63 ± 0.13deA
T	0.84 ± 0.02g	0.86 ± 0.01f	5.35 ± 0.14bD	10.11 ± 0.59aB	17.58 ± 0.15aA	9.39 ± 0.33bcC
W	0.98 ± 0.1fg	0.82 ± 0.03f	6.27 ± 0.18aC	7.67 ± 0.24bB	9.98 ± 0.60bA	9.93 ± 0.10bcA
C	2.55 ± 0.13a	1.16 ± 0.09c	4.85 ± 0.15cC	5.05 ± 0.52efC	6.75 ± 0.50dB	9.17 ± 0.28bcA
SS_T	1.02 ± 0.03f	0.73 ± 0.04g	5.18 ± 0.16bB	4.48 ± 0.07fB	5.08 ± 0.30eB	6.93 ± 0.67eA
SS_W	1.29 ± 0.04e	1.11 ± 0.02cd	4.44 ± 0.00dC	4.61 ± 0.20fC	6.15 ± 0.88deB	9.07 ± 0.63cA
SS_C	2.30 ± 0.16b	1.03 ± 0.03e	4.84 ± 0.12cD	5.37 ± 0.10deC	8.50 ± 0.29cA	8.03 ± 0.23dB
SS_T_W	1.00 ± 0.01f	0.84 ± 0.04f	2.86 ± 0.07fD	4.46 ± 0.23fC	5.38 ± 0.08eB	9.24 ± 0.50bcA
SS_C_W	1.63 ± 0.09d	1.32 ± 0.02a	4.84 ± 0.18cC	4.78 ± 0.15efC	8.63 ± 0.37bcB	10.52 ± 0.87bA
SS_C_T	2.05 ± 0.01c	1.24 ± 0.06b	4.09 ± 0.20eB	5.74 ± 0.16cdB	8.66 ± 2.13bcA	9.13 ± 0.26cA
SS_C_T_W	1.61 ± 0.13d	0.83 ± 0.05f	4.10 ± 0.28eC	5.95 ± 0.53cB	5.22 ± 0.38eB	11.38 ± 0.74aA

* SS data on WHC, OHC and S_p were presented in a previous work (Section B, Chapter 1).WHC, water holding capacity; OHC, oil holding capacity; S_p, swelling power; SS, sprouted sorghum flour; T, tapioca; W, wheat flour; C, cowpea flour; SS_T, sprouted sorghum and tapioca flour blend; SS_W, sprouted sorghum and wheat flour blend; SS_C, sprouted sorghum and cowpea flour blend; SS_W_T, sprouted sorghum, wheat and tapioca flour blend; SS_C_W, sprouted sorghum, cowpea and wheat flour blend; SS_C_T, sprouted sorghum, cowpea and tapioca flour blend; SS_C_W_T, sprouted sorghum, cowpea, wheat and tapioca flour blend. Values are expressed as mean ± SD (n=3). For S_p, values followed by different lowercase letters in each column are significantly different ($p \leq 0.05$). Values followed by different capital letter in each row are significantly different ($p \leq 0.05$).

3.3 Thermal properties

Except for T, which showed a unique thermal transition at 56-102 °C related to starch gelatinization, two endothermic peaks were evident in the other flour samples: the first one at ~52-96 °C and the second one at ~90-109 °C (Table 3). Among the three wheat-free blends, SS_C_T recorded the lowest onset temperature T_{on}, followed by SS_T and SS_C. The gelatinization event for W began at a lower temperature than the ones observed for all the other samples. W addition to the SS_C and SS_T blends (SS_C_W and SS_T_W) favoured the gelatinization decreasing T_{on}, while its presence in the SS_C_T_W blend caused no substantial difference on the onset of gelatinization.

Looking at the gelatinization enthalpies, SS_T showed the highest ΔH among the wheat-free blends due to the presence of T and its high percentage (50% w/w), followed by SS_C_T and SS_C. The addition of wheat to SS_C and SS_C_T flours and the concomitant reduction in the percentages of the other flours did not change the ΔH of gelatinization. Only for SS_T_W a strong increase in ΔH was recorded compared to SS_T, while an opposite effect was observed in the SS sample, since its ΔH decreased significantly after W addition.

Analysing the wheat-free blends' parameters, SS_C and SS_C_T flours showed a higher T_{on} but a lower ΔH than those recorded for SS_T, which can be explained by the presence of cowpea flour delaying the beginning of the transition and reducing its enthalpy.

By and large, the addition of W to the blends resulted in slight heterogeneous changes in the parameters associated with this thermal transition. In particular, the second peak's characteristic temperatures and enthalpies did not vary in a monotone way.

Table 3. Thermal properties of flours and their blends.

	First endothermic peak				Second endothermic peak			
	T_{on} (C°)	T_p (C°)	T_{off} (C°)	ΔH (J g^{-1})	T_{on}(C°)	T_p (C°)	T_{off} (C°)	ΔH (J g^{-1})
SS*	68.23 ± 0.18c	76.97 ± 0.16ab	86.12 ± 0.90cde	1.63 ± 0.31d	93.70 ± 1.34bc	98.67 ± 4.65bc	107.52 ± 0.93ab	0.33 ± 0.01a
T	56.45 ± 0.34d	71.64 ± 0.29d	102.37 ± 1.37a	9.30 ± 0.04a	n.a.	n.a.	n.a.	n.a.
W	53.73 ± 2.38e	67.19 ± 0.22e	74.53 ± 1.11f	3.34 ± 0.28c	90.74 ± 2.76de	97.89 ± 0.16bc	104.65 ± 0.84cd	0.34 ± 0.04a
C	72.83 ± 0.95ab	n.a.	95.62 ± 0.34b	1.53 ± 0.14d	96.87 ± 0.10a	102.52 ± 3.27a	105.74 ± 0.44c	0.06 ± 0.00e
SS_T	66.47 ± 0.52c	75.33 ± 0.39bc	85.05 ± 0.06e	3.12 ± 0.15c	92.61 ± 0.06cd	98.29 ± 0.44bc	103.95 ± 0.11d	0.22 ± 0.03bc
SS_W	74.25 ± 0.34a	79.08 ± 0.23a	85.53 ± 1.71de	0.42 ± 0.07e	91.00 ± 1.82de	97.55 ± 0.23c	104.77 ± 1.5cd	0.26 ± 0.05b
SS_C	71.68 ± 0.36b	78.33 ± 0.31a	96.27 ± 0.82b	1.69 ± 0.16d	96.90 ± 0.74a	100.90 ± 0.04abc	105.93 ± 0.70c	0.14 ± 0.00d
SS_T_W	52.03 ± 3.10e	72.98 ± 4.56cd	85.91 ± 0.36cde	5.72 ± 0.53b	90.20 ± 0.83e	97.97 ± 0.17bc	106.03 ± 0.91c	0.24 ± 0.08b
SS_C_W	57.55 ± 0.52d	n.a.	88.12 ± 0.66c	1.47 ± 0.06d	96.37 ± 0.59a	100.91 ± 0.19abc	106.17 ± 0.45bc	0.12 ± 0.01de
SS_C_T	58.07 ± 0.24d	73.58 ± 0.3cd	87.64 ± 0.91cd	2.12 ± 0.09d	96.61 ± 0.20a	101.08 ± 0.13ab	106.16 ± 0.59bc	0.11 ± 0.01de
SS_C_T_W	57.31 ± 0.12d	72.62 ± 0.14cd	84.57 ± 0.12e	2.11 ± 0.11d	95.41 ± 0.06ab	101.17 ± 0.34ab	108.86 ± 0.71a	0.17 ± 0.01cd

*SS data on thermal properties were presented in a previous work (Section B, Chapter 1). T_{on}, onset temperature; T_p, peak temperature; T_{off}, offset temperature; ΔH, peak enthalpy; SS, sprouted sorghum flour; T, tapioca; W, wheat flour; C, cowpea flour; SS_T, sprouted sorghum and tapioca flour blend; SS_W, sprouted sorghum and wheat flour blend; SS_C, sprouted sorghum and cowpea flour blend; SS_W_T, sprouted sorghum, wheat and tapioca flour blend; SS_C_W, *s*prouted sorghum, cowpea and wheat flour blend; SS_C_T, sprouted sorghum, cowpea and tapioca flour blend; SS_C_W_T, sprouted sorghum, cowpea, wheat and tapioca flour blend; nd; n.a., not available data. Values are expressed as mean ± SD (n=3). Values followed by different lowercase letters in each column are significantly different ($p \leq 0.05$).

3.4 Pasting properties

The pasting profile of SS, T, C and W samples is shown in Fig. S1, while the pasting properties of the flours and their blends are presented in Table 4.

Pasting temperature identifies the temperature at which an initial increase in viscosity occurs: the lower the pasting temperature, the lower the energy required to trigger the gelatinization event, which can be advantageous in baking. Among the wheat-free blends, SS_C showed the highest pasting temperature, in agreement with the higher gelatinization onset temperature observed in DSC. On the contrary, SS_T showed the lowest pasting temperature, remarkably close to the one recorded for W. The addition of W caused heterogeneous effects on pasting temperatures.

The peak viscosity represents the highest viscosity value reached during the heating cycle. As expected, the T sample showed the highest peak viscosity value (Table 4 and Fig. S1). Thus, considering only non-W flours, the two blends including T showed the highest viscosities, which were similar to that recorded for W. For both, the addition of W to the blend and the consequent decrease in the other ingredients determined a decrease in maximum viscosity, thus worsening the technological performance when high viscosity is required.

Peak temperature measures the temperature recorded at the maximum viscosity value. In the SS_T blend, the high amount of T, which was characterized by the lowest peak temperature value, resulted in maximum viscosity at a lower temperature than the other samples. The addition of W to the blend determined an increase in this parameter, which was ultimately comparable to that recorded for W flour.

For all other blends, the presence of C delayed the peak viscosity. Also in this case, the addition of W to the blends caused slight heterogeneous variations in the parameter considered.

Furthermore, the breakdown value provides information on the heat-stability of the starch paste. Considering the three wheat-free flour blends, the highest breakdown values were recorded when T was a part of the blend, while SS_C was found to be the most heat stable. The addition of W increased the heat stability of SS and the T-based composite flours, i.e., SS_T and SS_C_T.

With regard to the wheat-free samples, SS_C_T and SS_T showed a final viscosity of around 500 BU, which is lower than wheat but higher than all the other wheat-free blends. The setback value gives an indication of the retrogradation tendency of amylose in a starch paste. Among the samples not including W, SS_C recorded the lowest setback value, which is to be expected, given the high presence of cowpea. Low setback values indicated a low rate of starch retrogradation and syneresis, which would contribute to the maintenance of a soft structure during bread storage (Marti et al., 2017). Furthermore, the data showed that the use of W changed the setback in a heterogeneous way.

Table 4. Pasting properties of flours and their blends.

	Pasting temperature (°C)	Peak viscosity (BU)	Peak temperature (°C)	Final viscosity (BU)	Breakdown (BU)	Setback (BU)
SS*	76.45 ± 0.07c	92.50 ± 7.78f	89.90 ± 2.69d	105.00 ± 15.56g	47.50 ± 9.19f	59.00 ± 24.04h
T	64.15 ± 0.21f	1219.50 ± 16.26a	71.80 ± 0.57f	2186.00 ± 15.56a	719.00 ± 12.73a	1849.50 ± 33.23a
W	59.90 ± 1.80g	341.50 ± 17.50c	90.35 ± 0.05d	610.50 ± 32.50b	97.50 ± 1.50e	368.50 ± 9.50b
C	81.25 ± 0.05b	153.50± 0.50f	95.00 ± 0.00ab	241.00 ± 1.00f	10.50 ± 0.50h	98.00 ± 1.00g
SS_T	66.53 ± 0.55e	347.00 ± 23.64c	87.50 ± 0.85e	511.33 ± 48.34c	192.00 ± 17.69c	369.00 ± 34.77b
SS_W	85.5 ± 0.56a	177.00 ± 2.00e	95.23 ± 0.21ab	411.33 ± 4.04d	31.00 ± 1.73g	264.67 ± 8.50d
SS_C	85.07 ± 0.65a	166.67 ± 1.15ef	95.67 ± 0.12a	211.00 ± 4.36f	47.00 ± 1.73f	91.33 ± 3.06g
SS_T_W	66.35 ± 0.35e	277.5 ± 3.54d	89.50 ± 0.28d	397.50 ± 26.16d	102.00 ± 14.14e	209.00 ± 24.04e
SS_C_W	81.70 ± 0.30b	182.00 ± 1.00e	94.10 ± 0.60bc	301.67 ± 2.08e	58.33 ± 1.15f	181.33 ± 3.21f
SS_C_T	70.90 ± 2.30d	399.50 ± 9.50b	94.20 ± 0.80bc	522.00 ± 40.00c	207.00 ± 7.00b	329.50 ± 1.50c
SS_C_T_W	71.27 ± 0.45d	355.67 ± 0.58c	93.43 ± 0.55c	527.67 ± 3.51c	160.00 ± 1.73d	332.00 ± 4.36c

*SS data on pasting properties were presented in a previous work (Section B, Chapter 1).Pasting temperature, temperature at which an initial increase in viscosity occurs; Peak viscosity, maximum viscosity achieved during the heating cycle; Peak temperature, temperature at the maximum viscosity; Final viscosity, viscosity at the end of the test; Breakdown, viscosity difference between peak and after holding at 95 °C; Setback, difference between the final viscosity at 30 °C and the viscosity after the holding period at 95 °C; SS, sprouted sorghum flour; T, tapioca; W, wheat flour; C, cowpea flour; SS_T, sprouted sorghum and tapioca flour blend; SS_W, sprouted sorghum and wheat flour blend; SS_C, sprouted sorghum and cowpea flour blend; SS_W_T, sprouted sorghum, wheat and tapioca flour blend; SS_C_W, *s*prouted sorghum, cowpea and wheat flour blend; SS_C_T, sprouted sorghum, cowpea and tapioca flour blend; SS_C_W_T, sprouted sorghum, cowpea, wheat and tapioca flour blend. Values are expressed as mean ± SD (n=3). Values followed by different lowercase letters in each column are significantly different ($p \leq 0.05$).

3.5 Principal component analysis (PCA)

The first two components (PCs) explained 66% of the total variance (52% and 14% for PC1 and PC2, respectively; Fig. 1). PC1 was explained by T_{on}_ALC, T_p_ALC, peak T, ash, protein and carbohydrates, whereas PC2 was explained by T_{off}_gel, WHC, OHC, Sp_80 and the MVAG parameters peak viscosity, breakdown, setback, and final viscosity. The score plot of the first two principal components (t1/t2; Fig. 1a) highlighted PC1 enabled a clear discrimination of the T sample (left quadrant) from all the other flours, grouped at the centre of the plot. All the composite flours were distributed along PC2 on the basis of their greater or lesser similarity to the W and C flours which were located, respectively, in the negative and positive quadrants of PC2 at the extremes of the grouping, thus showing their opposite properties. Instead, SS was positioned in the negative quadrant of PC2, mingling with the composite flours and therefore showing intermediate behaviour with respect to the extremes of C and W, even if more similar to the latter. In addition to SS, in the negative quadrant identified by PC2, moving from sample W towards the centre of the plot, they were located the blends SS_T, SSD_T_W, SS_W and SSD_C_T_W, which gradually show intermediate behaviour between W and C. Meanwhile, in the upper quadrant, moving from the end of the group (C) towards the centre of the axes, they were located the C-based blends: SS_C, SSD_C_T and SS_C_W, with technological behaviour increasingly different from C and intermediate between C and W.

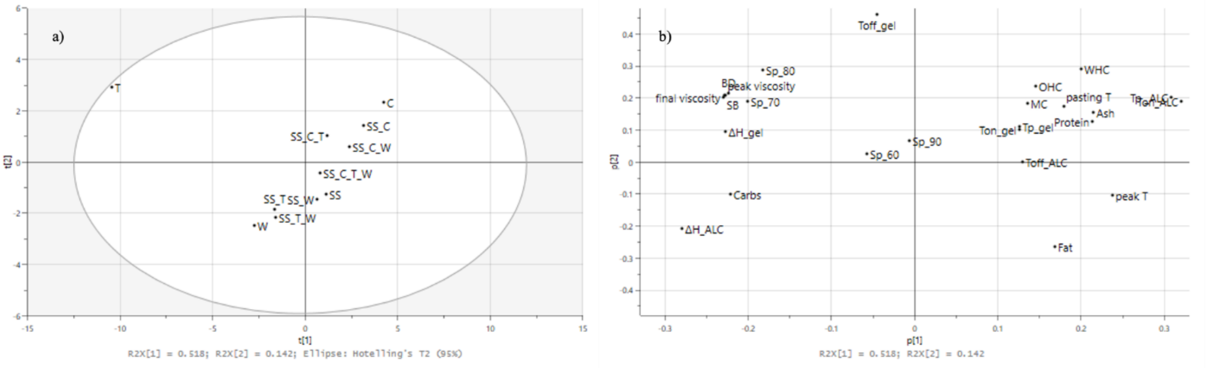

Fig. 1. Plots of the PCA model: a) score plot (t1/t2) and b) loading plot (p1/p2) of the first two principal components.

4. Discussion

The aim of this study was to assess the technological properties of sustainable and potentially high nutritional value composite flours to be used in the development of bakery products primarily targeted to African countries (i.e., flatbread). Therefore, sorghum, cowpea, and tapioca were chosen since they are staples and the main source of energy for people living in such regions. Since sprouting (or germination) is a traditional food processing in most sorghum-producing countries able to enhance the sensory and nutritional profile of the final product, flour from sprouted sorghum was used in the present study. Moreover, the synergistic effect of locally available crops was assessed on the functional and nutritional properties of both wheat-free and wheat-based blends.

Considering the chemical composition of the blends (Table 1), specific nutritional goals can be achieved in combining the various ingredients. Specifically, SS_T should be considered the best choice when a high content of readily available carbohydrates (Abass et al., 2016) is requested, whereas SS_C or SS_C_W when high content in high-quality proteins (Boye, Zare and Pletch, 2010), fat and ash is requested.

Once the nutritional value of the blends was assessed, their functional properties were studied by investigating the interactions of their proteins with water and oil.

WHC, as defined by the amount of water that can be held per gram of protein, is an important parameter in breadmaking functionality, given that high WHC of the dough is related to improved bread's properties (Jarpa-Parra, 2018; Ma et al., 2011). The WHC of the composite flours reflects the WHC of the single raw materials (Table 2), with C showing the highest value among them. Consequently, SS_C showed the highest value among the composite flours, suggesting the need of adding a high amount of water to achieve a good hydration degree during breadmaking. On the other hand, if the brief is a blend with WHC similar to that of W, then SS_T is recommended (and possibly also SS_T_W).

In bakery applications, the protein-oil interaction, as evaluated by OHC, is an important functionality for flavour retention, palatability, and shelf-life extension of finished products (Adebowale & Lawal, 2004). From the overall comparison of the flours, the best capacity to hold oil was seen in SS_C_W flour (Table 2); however, SS_C_T flour is particularly recommended, in light of its exceptionally good ability to hold oil coupled with the absence of wheat flour. Conversely, if the brief is a blend with OHC similar to that of wheat, then SS_T_W and SS_C_T_W should be chosen. Overall, besides the fundamental nutritional value as discussed above, the proteins in C seemed to exhibit an excellent capacity to hold water and oil, which makes it a potential raw material for breadmaking.

Swelling power, as well as pasting and thermal properties, were investigated to provide information about flour behaviour during processing (i.e., heating), which is related to the amount and structure of starch phase. S_p measures the water absorbed and entrapped within the starchy gel network during heating and stirring in excess of water (Li et al., 2014). S_p is influenced by several factors, including botanic origin, amylose/amylopectin ratio, size, morphology, and ultrastructure of starch granules, cell wall intactness, starch lipid complexes, and amorphous and crystalline domains in starch granules (Wani et al., 2016). At low temperatures, thermal energy swells starch without disrupting its granules; greater thermal energy provided by a rise in temperature induces crystalline structure breakdown with an increase in S_p (Li et al., 2014). The drop in S_p observed for T at 90 °C has already been observed in the literature, and it has been related to the leaching of cloudy solids caused by heating (Li & Yeh, 2001). Overall, SS_C_T seemed to be the composite flour with the best swelling capacity among the other blends, and its swelling profile with the increase in temperature seemed to be the most similar to the one recorded for W (Table 2). A greater swelling capacity was expected for SS_T given the higher presence of tapioca in the blend. Greater swelling capacity has been documented in the literature for T starch compared to cereals and legumes due to the different nature of the starch, the high percentage of amylopectin (i.e., the primary starch component responsible for S_p), and a low interaction of starch with other components hindering the swelling of the starch granules (Zhu, 2015). In SS_T sample, the effects of starch-protein-fibre interactions on S_p is worthy of interest.

Furthermore, also processing affects this parameter; indeed, the low S_p of SS has previously been related to the effects of sprouting on starch structure and on the accumulation of dextrins, oligosaccharides, and fermentable sugars limiting starch to form a compact gel (Singh et al., 2017; Section B, Chapter 1).

Thermal properties are mostly determined by the starch characteristics, granule size distribution and relative crystallinity (Ai, & Jane, 2015). For all samples (except for T) two endothermic peaks were evident: the first endothermic event (~52-96 °C) represented the starch gelatinization, whereas the second transition (~90-109 °C) was due to the dissociation and/or melting of crystalline amylose-lipid complexes (ALCs), which are typical of cereal starches and are either absent or present in exceedingly small amounts in tuber and pulse starches (Ai, & Jane, 2015; Bajaj, Singh, Kaur, & Inouchi, 2018). Concerning the starch gelatinization transition, its onset temperatures give an indication of the heat stability of crystalline starch structure with heating (Dhital, Shrestha, Hasjim, & Gidley, 2011). Higher transition temperatures are generally associated with higher starch crystallinity, which provides structural stability and makes granules more resistant to gelatinization (Bajaj et al., 2018). Moreover, gelatinization enthalpy reflects the energy intake for the dissociation of crystalline double-helices in starch granules (Ai, & Jane, 2015).

The shift to a higher gelatinization temperature observed in SS_C may be related to the higher presence of C in this blend (50% w/w), which suggests that higher energy may be required to initiate starch gelatinization. The different gelatinization properties of cereals *vs.* pulses have already well been documented in the literature (Ai, & Jane, 2015; Bajaj et al., 2018) and are attributable to several starch factors including crystallinity, granule size, and intermolecular bonding.

Overall, where gelatinization temperatures similar to those of W are required, the use of the blend SS_T_W is suggested. Otherwise, that is where other flours are included in the blend, higher gelatinization temperatures are required.

A Pearson correlation analysis highlighted a relationship between the flours' fat content and the ΔH of the ALCs melting peaks. Values of correlation coefficients (r) revealed that a higher fat content corresponded to a higher ΔH of the amylose-lipid complexes melting transition ($r=0.52$; $p < 0.05$).

By decreasing starch digestibility, ALCs are considered resistant starches and are thus a form of dietary fibre (Panyoo, & Emmambux, 2017). As a result, blends beginning the ALCs melting at a low temperature and presenting low ΔH values should be chosen to produce foods for undernourished people who require a readily available source of energy. Considering that all the blends showed significantly lower enthalpies than wheat, SS_T first of all, and then SS_W and SS_T_W, should be preferred to others.

Finally, the effect of temperature on starch properties was assessed by measuring the changes in viscosity during heating and cooling steps. Overall, the composite flours showed a better pasting profile than the single flours, i.e., more similar to that recorded for W and that have been correlated to good bread-making performances (Zi et al., 2019). For example, SS and C showed poor and overall lower pasting properties than W. Indeed, sorghum protein structures and their strong interactions affect starch functionalities (Hamaker, &

Bugusu, 2003) and sprouting worsen sorghum starch gelatinization behaviour (Marengo et al., 2015; Section B, Chapter 1). Moreover, the lower pasting properties of C than W are due to its botanical origin (Ai, & Jane, 2015). Consequently, the poor ability to generate viscous suspensions of these two flours is reflected in the derived blends. Given the excellent gelatinization capabilities, in all cases the addition of T is recommended to improve the pasting properties of composite flours including SS and C.

Finally, Principal Component Analysis (PCA) was performed to thoroughly understand the overall effect of the individual flours on the technological profile of the blends, and how they differ from one another from physicochemical and technological points of view. On the whole, the information from the PCA was poor, due to heterogeneous effects of different flours on technological parameters considered. However, given the proximity to sample W in the factorial space created by PC1 and PC2, SS_T would seem to be the only wheat-free composite flour to present technological behaviour remarkably similar to wheat. As such, it is expected to exhibit a technological performance comparable to W when used in the production of bakery products which do not require the viscoelastic properties of gluten. However, from a purely nutritional point of view, the inclusion of C is highly recommended to provide protein and microelements, as discussed above. If, on the one hand, the SS_C_T blend is positioned in the positive quadrant and therefore appears to show properties more similar to C, the SS_C_T_W flour is positioned in the exact centre of the score plot, thereby showing intermediate behaviour between the two extremes, and therefore appearing as a valid compromise between technological (W, T) and nutritional quality (C).

5. Conclusions

This work evaluated the physicochemical and technological properties of composite flours for the production of sustainable and nutritionally enhanced bakery products (e.g., flatbread) primarily targeted to African countries.

Sprouted sorghum flour is a widely used raw material in Africa and sprouting has proved to be a sustainable approach to improve its nutritional profile, although causing a worsening of the technological functionality of the starch component. Tapioca, on the contrary, exhibits excellent starch functional properties, which make it a locally available raw material indicated for improving the technological properties of sprouted sorghum flour. PCA output revealed that when used together to make a 50% w/w blend (SS_T), this composite flour exhibited technological properties which are by and large similar to wheat for considered parameters. However, the use of cowpea flour in breadmaking is recommended to provide protein and micronutrients and thus improve the nutritional profile of food products daily consumed in Africa. If sprouted sorghum, cowpea and tapioca blend (SS_C_T) represents an ideal composite flour from a nutritional and sustainability point of view given that it is entirely composed of local raw materials, the PCA analysis showed that the composite flour made up of sprouted sorghum flour, tapioca, cowpea flour and wheat flour at 25% w/w (SS_C_T_W) could represent a sound compromise between the flour's technological and nutritional quality, while helping to reduce the use of imported wheat flour as well as cutting cassava post-harvest losses. Indeed, when cassava roots have been harvested, they start deteriorating in just a few days, becoming worthless for consumption or industrial applications unless processed to lengthen shelf-life. Further studies would be necessary to investigate the use of SS_C_T_W composite flour in breadmaking by evaluating the technological, nutritional and sensory properties of the finished product, while evaluating a possible modulation of the formulation to produce sustainable flatbread with satisfactory technological and organoleptic properties and an improved nutritional profile.

Supplementary Material

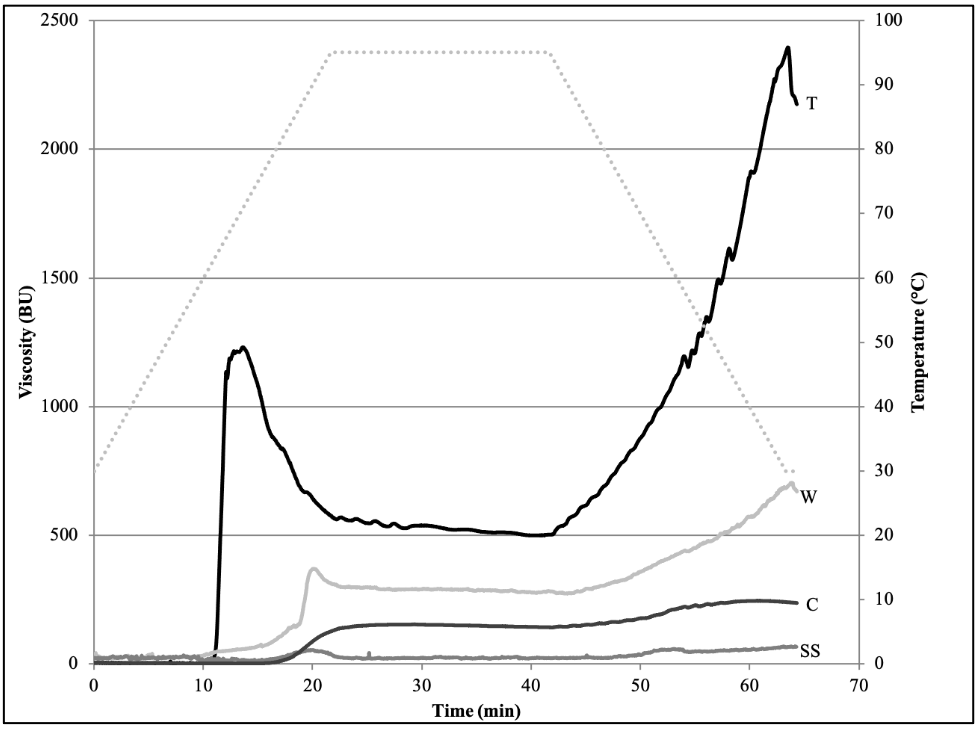

Fig. S1. Pasting properties of SS (sprouted sorghum flour), C (cowpea flour), W (wheat flour) and T (tapioca) samples measured by means of a Micro-Visco Amylograph. SS pasting profile was presented in a previous work (Section B, Chapter 1)

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Chapter 5

Towards sustainable and nutritionally - enhanced flatbread targeted to African countries

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Abstract

The use of composite flours in flatbread production, in which all or part of the wheat flour is replaced by locally grown crops, should be encouraged in African countries to decrease the use of imported wheat while producing nutritionally enhanced bread. The aim of this study was to develop nutritionally enhanced and economically sustainable flatbreads using composites of sprouted sorghum, tapioca, cowpea and wheat flours. Three flatbread prototypes, within the experimental set identified by the experimental design, were chosen on the basis of their textural (the most similar to the reference 100% wholewheat flatbread), nutritional (the highest intake of energy, proteins and micronutrients, such as Iron, Zinc and Vitamin A) and economic (the least expensive based on prices of raw ingredients in Sierra Leone, Tanzania, Burundi and Togo) features, and further characterized for their physicochemical properties, in vitro starch digestibility, total phenolic content, antioxidant capacity and sensory acceptability, in comparison to the control.

Overall, the partial substitution of wheat flour with sprouted sorghum, tapioca, and cowpea flours blended in different proportions resulted in some very interesting products which featured delayed starch hydrolysis and a higher total phenolic content and antioxidant activity, acceptable texture quality and high sustainability compared to the reference 100% wholewheat flatbread.

Keywords: Composite flours; Sustainability; Flatbread; Cowpea flour; Sprouted sorghum; Starch digestibility.

Abbreviations: SS, sprouted sorghum flour; T, tapioca; W, wholewheat flour; C, cowpea flour; alveographic parameters - W (J 10^{-4}), baking strength; P/L ratio, curve configuration ratio; P (mm), dough tenacity; L (mm), dough extensibility; d.b., dry basis; w.b., wet basis; FFD, fractional factorial design; R/T, room temperature; MC, moisture content; STD, 100% W flatbread; JAR, Just-about-right; PA, penalty analysis; S, flour from unsprouted sorghum; RDS, rapidly digestible starch; SDS, slowly digestible starch; DS, digestible starch; RS, resistant starch; TS, total starch; TPC, total phenolic content; GAE, gallic acid equivalents; DPPH•, 2,2-diphenyl-1-picrylhydrazyl radical; TEAC, Trolox Equivalent Antioxidant Capacity; Sd, standard deviation; ANOVA, analysis of variance.

1. Introduction

Developing bakery products using composite flours is currently of great interest to researchers, as it represents a way to improve the nutritional value of standard wheat-based products (Noorfarahzilah, Lee, Sharifudin, Fadzelly, & Hasmadi, 2014). Moreover, if the composite flour is obtained from both nutritious and sustainable (i.e., affordable) ingredients, the approach can be particularly efficacious for developing countries. This approach has the advantages to save money by reducing importation of wheat flour, provide better supply of macro and micronutrients in the diet and encourage a better use of local agriculture production by employing indigenous crops to produce flour (Noorfarahzilah, et al., 2014).

In the Middle East, North Africa, and Central Asia, bread has been a staple food for centuries, and its consumption is among the highest in the world, being eaten with almost every meal (Al-Dmoor, 2012; Noorfarahzilah et al., 2014). In these countries, the type of bread traditionally produced and consumed, especially at home, is flatbread, i.e., a simple unleavened bread made from a flattened piece of dough containing flour, water, salt and other optional ingredients (Al-Dmoor, 2012), and for whose production the exceptionally high viscoelastic performance of wheat is not required.

The use of composite flours for flatbread production, by a complete or partial replacement of wheat flour with locally grown crops, should be actively encouraged, to decrease the use of imported wheat while producing nutritionally enhanced bread (Noorfarahzilah et al., 2014).

Sorghum flour is extensively used in Africa and sprouting is a proven sustainable technique which improves its nutritional profile, albeit with a worsening of starch functionality (Xu, 2019; Singh, Sharma, & Singh, 2017). In contrast, tapioca starch presents excellent technological properties, which makes it a locally sourceable raw material recommended to improve the technological properties of sorghum flour (Zhu, 2015; Falade, & Akingbala, 2010; Abass et al., 2018). The use of cowpea flour for breadmaking is also advocated to provide proteins and bioactive compounds and improve the nutritional profile of foodstuffs consumed in Africa on a day-to-day basis (Kerr, Ward, McWatters, & Resurreccion, 2000; Da Silva, Da Costa Santos, Junior, Da Silva, Dos Santos, & Siviero, 2018; Jayathilake, Visvanathan, Deen, Bangamuwage, Jayawardana, Nammi, & Liyanage, 2018).

In a previous study (Section B, Chapter 4) the technological properties of sustainable composite flours made of sprouted sorghum, tapioca, cowpea, and wheat flours [in relative abundance to 50%, 33% and 25% (w/w) flour basis] were evaluated. The results of the Principal Component Analysis showed that, when used to produce a 50% w/w flour blend, sprouted sorghum and tapioca flours exhibited a technological profile similar to wheat flour, which may be of interest in bakery products development when the viscoelastic performances of gluten are not requested. However, a composite flour made from sprouted sorghum flour, tapioca, cowpea, and wheat flours was proved to be a reliable compromise between a flour's technological and nutritional qualities, while cutting both the use of imported wheat flour and cassava post-harvest losses (Section B, Chapter 4). Since the final quality of flatbreads is related to the technological and nutritional properties of the used flours, along with their percentage in the blend, there is the need to optimize the formulation, in order to improve the overall quality of derived finished products. In this regard, the aim of this study was to develop nutritionally enhanced, economic sustainable and texture acceptable flatbread formulations targeted to African countries. Formulations including composites flours made of sprouted sorghum, tapioca, cowpea and wheat flours, were characterized for their physico-chemical, nutritional and sensory features.

2. Materials and methods

2.1 Flours

Sorghum kernels were soaked (25 °C for 16 h), sprouted (25 °C for 72 h), dried (40 °C for 12 h) and milled (Labormill, BONA, Monza, Italy) to produce refined flour, middlings, and bran, which were recombined as wholemeal sorghum flour (SS). The processing conditions used to obtain flour from sprouted sorghum were chosen based on previous experiments (Section B, Chapter 1 and Chapter 2), which demonstrated as this process led to sorghum protein and starch hydrolysis, with a consequent increase in total amino acid content, starch digestibility, water and oil holding capacity. Moreover, the metabolic and enzymatic pathways activated during the sprouting process led to a decrease in phytic acid and an increase in vitamins, minerals, total polyphenol content, along with their soluble and bioaccessible fractions, and antioxidant activity, compared to unsprouted sorghum flour (Section B, Chapter 1 and Chapter 2). The proximate composition of SS flour was as follows (%, g/100 g dry basis): carbohydrates 83.6 ± 0.1%; proteins 12.0 ± 0.0%; fats 3.1 ± 0.0%; ashes 1.3 ± 0.0; moisture 12.7 ± 0% wet basis (w.b.).

Tapioca (T) and cowpea wholemeal (C) flours were purchased from Molino Bongiovanni S.r.l. (Villanova Mondovì, CN, Italy), while wholewheat flour (W) [W= 240 J 10^{-4} and P/L = 0.55; Italian legislation - (D.P.R. (Presidential Decree) 187, 2001)] was purchased from Molino Grassi S.p.A. (Fraore, PR, Italy).

2.2 Flatbread formulation, production, and characterization

2.2.1 Experimental design

An experimental fractional factorial design (FFD) with Resolution V was used to investigate the effect of SS, T, C and W levels (input factors, independent variables) on the textural properties of the derived flatbread (responses, dependent variables) when used in composite flours. The experimental FFD was chosen as a screening tool to evaluate the preliminary significance of the variables and their interactions, while reducing the number of experiments required and avoid loss of significant information. Water was included as an uncontrolled factor, since its use as a controlled/constant input had produced a set of unfeasible experiments in preliminary trials, i.e., unworkable doughs due to too low or too high hydration. A centre point and two levels (minimum and maximum) for each input factor were selected in preliminary screening tests to meet different criteria. Firstly, formulations had to include all four types of flour; secondly, SS and C flours had to be included as much as possible and W as little as possible while ensuring the dough workability for flatbread production. The levels for the experimental design and the experiment compositions (F1-F9) are reported in Table 1.

Table 4. Experimental design of composite flour formulations for flatbread development. In brackets. the percentage of flour ingredient expressed on 100 g of composite flour.

		X_1	X_2	X_3	X_4	X_5
Exp. name	Run order	W (g)	T (g)	SS (g)	C (g)	Water (mL)
F1	6	22 (11.2)	22 (11.2)	75 (38.1)	78 (39.7)	112.7
F2	5	32 (14.7)	32 (14.7)	75 (34.6)	78 (36)	110.8
F3	10	32 (12.5)	22 (8.6)	125 (48.6)	78 (30.3)	146.1
F4	8	22 (8.6)	32 (12.5)	125 (48.6)	78 (30.3)	147.8
F5	7	32 (12.5)	22 (8.6)	75 (29.2)	128 (49.7)	142.6
F6	11	22 (8.6)	32 (12.5)	75 (29.2)	128 (49.7)	130.8
F7	4	22 (7.4)	22 (7.4)	125 (42.1)	128 (43.1)	161
F8	9	32 (10.1)	32 (10.1)	125 (39.4)	128 (40.4)	183.3
F9	1	27 (10.5)	27 (10.5)	100 (38.9)	103 (40.1)	140
F10	2	27 (10.5)	27 (10.5)	100 (38.9)	103 (40.1)	140
F11	3	27 (10.5)	27 (10.5)	100 (38.9)	103 (40.1)	140

Levels of W and T = 22. 27 and 32 g; levels of SS = 75. 100 and 125 g; levels of C = 78, 103 and 128 g.

The three-replicate centre points (Exp. F9-F11) allowed the calculation of experimental error in the analyses and predicted whether the experimental design would give significant lack-of-fit (Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007).

The chosen responses were the following: the textural flatbread quality attributes force at rupture (f, N) and extensibility (e, mm), measured with a puncture test (P) and a one-dimensional extensibility test (E), respectively (TA.XT2 Texture Analyzer, Stable Micro Systems, Goldalming, UK); moisture content (MC, g water/100 g sample); water activity (a_w).

2.2.2 Flatbread production

Flatbreads were produced as follows: the dry ingredients were mixed at room temperature (R/T) in a home bread-maker (Backmeister 68511, UNHOLD, Germany) for 2 min, then sunflower seed oil and salt (2% w/w) were added and mixed for further 2 min, and finally distilled water was added in variable quantities (Table 1) until a dough with an optimal consistency (empirically evaluated) similar to that of the centre point was obtained. After a resting period of 5 min at R/T, the dough was manually shaped into 50-g balls which rested for an additional 25 min at R/T, then they were laminated twice (Nonna Pasta 180, Kasaviva, TI, Italy) to obtain a circle of dough (1.85 ± 0.12 mm thickness). The shaped pieces of dough were then cooked at 200 °C on a glass-ceramic skillet (Schott Ceran, Germany) for 1 min per side. The flatbread was left to cool at R/T prior to analysis.

2.2.3 Texture

The flatbread's textural properties were determined by means of a texture analyser (TA.XT2 Texture Analyser equipped with a 25-kg load cell, Stable Micro Systems, Goldalming, UK) using a puncture test and a one-dimensional extensibility test, following the methods described by Bejosano and colleagues (Bejosano, Joseph, Lopez, Kelekci, & Waniska, 2005). Rupture force (N) and rupture distance (mm) were recorded for both tests. At least 5 replicates were performed for each test for each flatbread prototype.

2.2.4 Moisture content (MC) and water activity (a_w)

Moisture content was measured by weight loss after forced-air oven drying at 105 °C at constant weight (ISCO NSV 9035, ISCO, Italy). Analyses were performed in triplicate.

Water activity was measured at 25 °C using an Aqualab 4 TE (Decagon Devices, Inc., USA). Samples were divided into small pieces immediately before analysis. A minimum of 6 determinations were carried out for each flatbread prototype.

2.3 Choice of formulations: technological, nutritional and economic criteria

Since the experimental design generated weak models, characterized by high mathematical uncertainty and low predictivity (see Paragraph 3.1), preventing us from drawing firm conclusions and from choosing the best formulation from a technological point of view, we decided to produce a 100% W flour-based flatbread to take as a reference (STD) to evaluate textural properties of composite flour flatbreads. W flatbread was prepared by mixing 265 g of flour with 187.5 mL of water following the recipe described above (Paragraph 2.2.2).

Consequently, to meet the objective of this work, i.e., to develop (i) nutritionally enhanced, (ii) economically sustainable and (iii) quality acceptable flatbreads using blends made of as much as possible SS and C flours and as little as possible W, the formulations originated by experimental design were evaluated based on the textural properties of derived flatbreads, estimated nutritional value and costs, following the methods described below.

The formulations that showed the best performance for these criteria were selected for further characterization.

- *Physico-chemical properties*

Textural properties (P_f, P_e, E_f; E_e) of flatbreads were compared with those measured for the STD flatbread. The formulation having textural properties more similar to those recorded for STD was considered eligible to satisfy the technological criterion and therefore selected for further characterization.

- *Nutritional value*

A nutritional evaluation of the studied formulations was carried out. Firstly, the composition in macro and micronutrients expressed on 100 g of flour was calculated on the basis of nutritional information obtained analytically on SS (Paragraph 2.1), integrated with information obtained from the FAO / INFOODS Food Composition Table for Western Africa 2019 (Vincent et al., 2020). Then, for each nutrient, the range of variation within the experimental set identified by the experimental design (F1-F9) was established. For each range and for each nutrient, 4 target levels were defined, i.e.: minimum intake, intake equal to 33% of the range, intake equal to 66% of the range and maximum intake. Lastly, for each nutrient of each formulation, the following scores were assigned: 0 = an intake between the minimum value and ≤ 33% of the range of variation; 1 = an intake > 33% but ≤66% of the range; 2 = an intake > 66%. The individual scores were added up to obtain the final score for each formulation. However, since the most prevalent nutritional deficits among females in sub-Saharan Africa were estimated to be protein-energy malnutrition and Iron, Zinc and Vitamin A deficiencies (Leslie, Ciemins, & Essama, 1997; Lartey, 2008), the weight of the scores obtained for these components was considered double. To conclude, the individual scores were added up and the formulation with the highest total score was considered eligible to satisfy the nutritional criterion and therefore selected to further characterization.

- *Economic sustainability*

The economic sustainability of the formulations was evaluated by calculating their prices ($/kg) in different African countries as Sierra Leone, Tanzania, Burundi and Togo, based on the costs of the single raw materials and their % of addition in the blend. These information were collected on-site by local partnership institutions and collaborators of the University of Parma. The least expensive formulation answered the economic sustainability criterion was selected for further analysis.

2.4 Characterization of selected flatbreads formulations

2.4.1 Colour analysis

Colour was analysed with a CIELAB colorimeter (CM 2600d, Minolta Co., Japan) under standard illuminant D65. $L*$ (Lightness), $a*$ (degree of redness) and $b*$ (degree of yellowness) parameters were measured using a 10° position of the standard observer. At least 15 determinations were taken by analysing the surface of three flatbread samples for each prototype. Differences in colour between flatbread prototypes and STD sample were evaluated using the ΔE value, calculated following [Eq. 1]:

$$\Delta E = [(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}]^{1/2} \qquad [1]$$

2.4.2 Sensory analysis

Consumers' sensory evaluation of flatbreads was assessed with an acceptability test, a preference ranking test, and a Just-About-Right (JAR) test. The flatbreads were produced few hours before the analysis. After baking and cooling, discs with a 5-cm diameter (Fig. S1) were obtained using a pastry cutter, divided in half and identified with random three-digit codes. Samples were simultaneously presented on a plate in randomized order and in blind conditions; consumers were asked to taste and evaluate one sample at a time. Water was served to cleanse the palate between tastings. The panel consisted of 27 untrained African consumers, of which 17 were students at the University of Parma and 10 were recruited among workers of a local farm. The panel was limited in number due to the difficulty to recruit a larger number of consumers during the COVID-19 emergency period. Indeed, the consumers were interviewed before the lockdown established in Italy in November 2020. Before the analyses, the consumers were asked questions about their age, gender and African State of origin. At first, the panelists evaluated the acceptability of the flatbread by rating overall acceptability, consistency, appearance and aroma by means of a 9-point hedonic scale [1=dislike extremely, 2=dislike very much, 3=dislike, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like, 8=like very much and 9=like extremely]. Then, for the JAR test, consumers rated the samples on a 5-point JAR scale (1=much too low, 2=too low, 3=just about right, 4=too much, and 5=far too much) for hardness, darkness, bitterness and bean flavour. After tasting all the samples, consumers were asked twice to answer a preference ranking test, before and after providing them a brief explanation of the project and samples.

2.4.3 Relevant starch nutritional fractions

Relevant starch nutritional fractions defined, based on *in vitro* starch digestibility, as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), were quantified by means of the Megazyme Digestible Starch and Resistant Starch assay procedure (K-DSTRS 11/19 commercial kit, Megazyme International Ireland Ltd., Wicklow, Ireland) following the manufacturer's protocol based on the method described by Englyst, Vinory, Englyst, & Lang (2003).

Two batches of each bread type were produced in different days, each one analysed in triplicate. Six determinations of RDS, SDS and RS fractions, respectively, were acquired. Digestible starch (DS, RDS+SDS) and total starch (TS, DS+RS) were also calculated.

2.4.4 Total polyphenol content (TPC) and DPPH• free radical scavenging activity analysis

Flatbread extracts were prepared starting from 1 g of ground bread, added to 20 mL of a methanol/water (70:30 v/v) mixture, extracted on a stirrer at R/T for 1 h and then filtered using a filter paper. The solvent was evaporated, and the extract was redissolved in 1 mL of a methanol/water (70:30 v/v) mixture and centrifuged at 5040 x g (6255 rpm) for 15 min at 4 °C.

For the total phenolic content (TPC) measurement, 50 μL of each extract was mixed with 1160 μL of distilled water, 300 μL of Na_2CO_3 aqueous solution (20% w/v) and 100 μL of Folin–Ciocalteu's reagent. The absorbance of the solution was measured at 760 nm by an UV- Visible spectrophotometer (JASCO V-530 spectrophotometer, Easton, MD, USA) after 30 min of incubation in the dark at R/T.

A calibration curve using gallic acid as external standard (10-300 mg/L, 5 points) was prepared for quantification. The results were reported as mg of gallic acid equivalents (GAE) per kg of bread on d.b.

Antioxidant capacity was determined using a DPPH• assay (2,2-diphenyl-1-picrylhydrazyl free radical) following the procedure proposed by Dall'Asta, Cirlini, Morini, Rinaldi, Ganino, & Chiavaro (2013).

Briefly, 200 μL of each extract was mixed with 2.6 mL of methanol and 2 mL of a methanolic DPPH• solution (0.2 mmol/L). The absorbance of the solution was measured at 517 nm using a JASCO V-530 spectrophotometer (Easton, MD, USA) after 30 min of incubation in the dark at R/T. Blank was prepared and analysed following the same procedure. The radical scavenging activity was calculated as follows [Eq. 2]:

$$I\% = [(Abs_0 - Abs_1)/Abs_0] \times 100 \qquad [2]$$

where Abs_0 and Abs_1 were the absorbance of the blank and of the sample, respectively.

TEAC value (Trolox Equivalent Antioxidant Capacity; μmol Trolox eq./g of bread on d.b.) was obtained from the 0.1 – 0.5 mmol/L calibration curve calculated measuring the absorbance at 517 nm of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) methanolic solutions at different concentrations. Two batches from each bread type were analysed and the analyses were performed in duplicate.

2.5 Statistical analysis

All data were expressed as mean ± standard deviation (SD).

MODDE 12.1 Software (Umetrics AB, Malmö, Sweden) was used to design the experiments and also to analyse the outcomes. The experiment results were analysed statistically by the PLS method. Model validity and reproducibility [i.e., a variation in the response under the same conditions (pure error), often at the centre points, compared to the total variation of the response) were also evaluated]. To interpret the influence of terms (factors) on each model, the Coefficient Plot and Effect Plots and lists were evaluated. Each model was refined by removing outliers and/or insignificant terms and/or adding significant/interaction terms. The reliability of the models was then evaluated by calculating the R^2 and Q^2 values, where R^2 is the percentage of the variation in the response explained by the model and Q^2 is the percentage of the variation in the response predicted by the model according to cross validation, expressed using the same units as R^2.

One-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test at 0.05 significance level (SPSS Statistical Software, Version 25.0, IBM SPSS Inc., USA) were performed to assess significant differences between the samples.

A Friedman Nonparametric test followed by multiple pairwise comparisons using Nemenyi's procedure was used to determine the significance of the ranking preferences. For JAR data analysis, firstly, the impact of the JAR variables on the overall liking scores was calculated using Spearman's correlation coefficient. Then, Penalty Analysis (PA) was performed as follows: (i) grouping the 5-point JAR scale into 3 levels (too little, JAR, and too much) for each attribute; (ii) calculating the mean overall liking score obtained from the hedonic-scale scores and the percentage of respondents represented in each of the 3 categories; (iii) calculating mean drops by subtracting the mean overall liking score for the JAR group from the mean overall liking score of the "too much" or "too little" categories (Zhi, Zhao, & Shi, 2015). The penalty was then calculated as a weighted difference between the means (mean of liking for JAR - mean of liking for the two other levels taken together). Ranking and JAR sensory data analyses were performed using XLSTAT Software (Version 2020.3.1, Addinsoft, New York, USA).

3. Results and discussion

In a previous work (Section B, Chapter 4), a composite flour made up of SS, T, C and W at 25% w/w flour basis (f.b.) was proved to offer a good compromise between technological and nutritional qualities. Then, the aim of this work was to investigate the use of SS_C_T_W composite flour in breadmaking by evaluating the physico-chemical, nutritional and sensory properties of the finished product, as to evaluate a possible modulation of the formulation to enhance the sustainability, texture and nutritional quality of flatbread formulations.

After preliminary trials carried out by increasing the levels of SS, C and T (locally sourced raw materials), lowering the level of W (imported flour in Africa), and evaluating the workability of the derived dough, an experimental design was set for screening purposes to investigate the effect of SS, T, C and W levels on the textural properties of the derived flatbreads.

3.1 Experimental design

The experimental design allowed to program experiments useful to homogeneously test a domain defined by 4 controlled factors (SS, T, C, W), i.e., one uncontrolled factor (water), i.e., and 5 dimensions.

Very low reproducibility was found for MC and a_w responses, which means that by changing the experimental conditions, they do not vary sufficiently with respect to the variability of the repeated measurement data. The variation of the data obtained for the two responses was overall comparable with the experimental error determined on three-replicate centre points, and for this reason their analysis was not investigated further.

For all the other responses, however, the reproducibility of the data was sufficient to further study the models obtained though they resulted weak and characterized by high mathematical uncertainty. For all the answers, the effects of the factors are to be read in probabilistic terms.

The model generated for the force (N) measured with puncture test (P_f response) was characterized by an $R^2 = 0.9$ and a $Q^2 = 0.2$. A probable linear dependence of the response on the SS and C factors was observed: an increase in SS materialized in a probable negative effect on the response (decrease in P_f), while an increase in C was more likely to affect positively (increase in P_f). A negative effect from the interaction of the two factors (SS * C) was also possible.

The model generated for the P_e response, i.e., the extensibility (mm) measured with puncture test, was characterized by an $R^2 = 0.7$ and a $Q^2 = 0.5$. A probable linear dependence on factors T and W was observed: an increase in T materializes in a probable positive effect on the response (increase in P_e), while an increase in W more likely affected negatively (decrease in P_e). The interaction coefficients revealed a positive quadratic dependence of the response on the factors W and T (W * W and T * T) indicating the need for a second order equation to describe the model. There was also an interaction effect of the two factors (W * T), manifested with a distortion effect of the response surface.

The model generated for the force (N) measured with one-dimensional extensibility test (E_f response), was characterized by an $R^2 = 0.7$ and a $Q^2 = 0.2$.

A probable linear dependence on the factors SS, T and water was found: an increase in SS or T materialized in a probable negative effect on the response (decrease in E_f), while the increase in water was more likely to affect positively (increase of E_f). As before, the interaction coefficients revealed a positive quadratic dependence of the response on the factors SS and T (SS * SS and T * T).

Finally, the model generated for the extensibility (mm) measured with one-dimensional extensibility test (E_e response) was characterized by an $R^2 = 0.6$ and a $Q^2 = 0.5$. Only the factor SS exerted an influence on the response, and a negative linear dependence was identified for it (as SS increased, E_e decreased). Since a $Q^2 > 0.5$ is recommended to draw conclusions from a model (Flander et al., 2007), overall, the low predictivity of the models and the high uncertainty of the effects of the factors on the responses prevented us from drawing firm conclusions and using the mathematical equations describing the models to make predictions. For this reason, the formulations proposed by the experimental design, being all workable, have been evaluated on the basis of 3 criteria: textural quality, theoretically nutritional value, and economic sustainability.

3.2 Choice of formulations: technological, nutritional and economic *criteria*

- *Physico-chemical properties*

The textural properties, MC and a_w of flatbread samples are reported in Table 2.

Table 2. Experimental design results of composite flour formulations for flatbread development and comparison with textural properties of a 100% W flatbread (STD).

Trial	Puncture test		One - dimensional extensibility test		MC (g/100 g)	a_w
	P_f (N)	P_e (mm)	E_f (N)	E_e (mm)		
F1	3.84 ± 0.1e	11.45 ± 0.47abc	8.56 ± 0.39b	3.26 ± 0.53b	29.77 ± 0.34bcde	0.9087 ± 0.0013bcd
F2	2.38 ± 0.28f	10.26 ± 0.42cd	5.47 ± 0.8f	3.06 ± 0.46bc	29.34 ± 0.01de	0.9104 ± 0.0009bc
F3	4.78 ± 0.95cde	11.31 ± 1.95abc	7.54 ± 1.32bc	2.76 ± 0.6bc	30.93 ± 0.31b	0.9064 ± 0.0008cde
F4	2.92 ± 0.74f	12.81 ± 1.32a	5.98 ± 1.32ef	2.11 ± 0.63d	32.74 ± 0.68a	0.9184 ± 0.0041a
F5	5.37 ± 0.63bc	10.72 ± 1.05bcd	10.01 ± 0.73a	3.1 ± 0.51bc	30.03 ± 0.45bcd	0.9028 ± 0.0042de
F6	5.89 ± 1.13b	12.64 ± 0.37ab	7.43 ± 1.12bcd	3.23 ± 0.54b	29.57 ± 0.52cde	0.8964 ± 0.002f
F7	2.51 ± 0.52f	9.76 ± 1.34cd	6.88 ± 1.57cde	2.6 ± 0.38c	32.45 ± 0.47a	0.9173 ± 0.0003a
F8	3.99 ± 0.41e	11.41 ± 1.67abc	7.42 ± 1.6bcd	2.75 ± 0.54bc	33.3 ± 0.02a	0.9148 ± 0.0031ab
F9	4.99 ± 1.07bcd	9.95 ± 0.35cd	6.43 ± 1.22cdef	2.73 ± 0.5bc	26.1 ± 0f	0.884 ± 0.009g
F10	4.51 ± 0.84cde	9.29 ± 0.85d	6.25 ± 0.6def	2.64 ± 0.28c	29.52 ± 0.89cde	0.9021 ± 0.0036ef
F11	4.36 ± 0.38de	10.05 ± 0.73cd	7.12 ± 0.82cde	3.1 ± 0.31bc	28.66 ± 1.36e	0.9015 ± 0.0027ef
STD	7.78 ± 1.26a	12.91 ± 3.32a	6.12 ± 1.02ef	7.32 ± 0.6a	30.7 ± 0.28bc	0.9132 ± 0.0010ab

Values are expressed as mean ± SD ($n \geq 5$; n=3 for moisture content, MC). Values followed by different letters in each column are significantly different (one-way ANOVA with Duncan's post-hoc test. $p \leq 0.05$).

STD flatbread recorded the highest rupture force (~8 N) and elasticity (~13 mm) indicating a more cohesive and extensible structure probably due to the presence of higher amount of gluten proteins in this formulation if compared with flatbreads obtained by composite flours. Although significantly different ($p \leq 0.05$), sample F6 is the only one that comes closest to STD as regards P_f (~6 N) and, together with F4, also as regards P_e (~13 mm for both F6 and F4).

Data obtained from a one-dimensional extensibility test showed for the STD sample an intermediate rupture force (~6 N) if compared to the variation range identified for the other samples [~5 N (F2) - 10 N (F5)]. On the other hand, the extensibility of the STD (~7 mm) was significantly higher than all the other formulations; F2 and F6 were characterized by extensibility more similar to STD, although significantly ($p \leq 0.05$) lower (~3 mm for both samples).

Moisture content of STD was 30.7 g water/100 g sample, within the range identified by composite flour breads [from 26.1 (F9) to 33.3 g water/100 g sample (F8)]. Despite F1, F3, F5, F6 and F10 recorded similar MC if compared to STD, all of them showed significantly ($p \leq 0.05$) lower a_w, which may be related to stronger interactions between biopolymers and water in these samples, if compared with STD.

Overall, the changes in the textural attributes of flatbreads may be related to the different water-solids interactions developed as a function of a different composition (gluten and non-gluten proteins, starch, sugars, fibre, etc.) of the flours, and to their different percentage in the blend. Polymers of a different chemical and/or botanical nature can interact in a different way with water (Section B, Chapter 3) and compete for the formation of molecular bonds with it. Different interactions and different hydration levels of the polymers significantly affect the water state and distribution in complex matrices with inevitable repercussions on the texture attributes of the derived products (Serventi, Carini, Curti, & Vittadini, 2009).

Since sample F6 showed an intermediate E_f value (~7 N), being however all other textural attributes more similar to the STD, it was identified as the sample meeting the technological criterion of the highest textural quality.

- *Nutritional value*

Estimated proximate composition of the flatbread formulations is presented in Table 3.

An initial evaluation of the formulations was provided with scores (0, 1 or 2) based on the intake provided for each nutrient if compared to the range of variation within the experimental set. Based on this attribution (data not shown), the formulations F5, F6 and F7 recorded the highest score with regard to the contribution of energy, macro-components (8) and micronutrients (26, 28 and 30, respectively). Overall, F7 was evaluated as the formulation with the best nutritional profile (total score = 38), followed by F6 (total score = 36) and F5 (total score = 34). In accordance with previous findings which revealed that the most prevalent nutritional deficits among females in sub-Saharan Africa were protein-energy malnutrition and Iron, Zinc and Vitamin A deficiencies (Leslie et al., 1997; Lartey, 2008), in a second evaluation, greater relevance was attributed to the contribution of the aforementioned components by multiplying the respective scores by two. The results are reported in brackets in Table 3. Formulations F5 and F6 were given the highest rating (12) for the contribution of energy and macro-components, followed by F7 (10), which differs from the other two mainly for the lower energy intake. The highest score for micro components, on the other hand, was attributed to F7 (36), followed by F6 (34) and F5 (32). Overall, F6 and F7 were evaluated as the formulations with the best nutritional profile (total score = 46) thus responding to the nutritional criterion, followed by F5 (total score = 44). Their highest nutritional profile was probably the result of their high nutrients rich flour content (e.g., SS and C made up more than 85% flour in F7) (Jayathilake et al., 2018; Da Silva et al., 2018; Section B, Chapter 1 and Chapter 2). Comparing F6 and F7 with STD, the formers provided a higher intake of proteins and most micronutrients than the latter, along with a lower intake of energy, carbohydrates, fat, fibre, iron, phosphorus and Vitamin B6. As a result, F6 and F7 were selected for further analysis on the basis of the nutritional criterion. Interestingly, F6 was already chosen given its compliance with the technological criterion.

Table 3. Estimated macro and micronutrient composition of flatbread formulations per 100 g flour. In brackets, the score attributed for the intake of each nutrient provided by the formulation. The total score is the sum of the partial scores.

Component (100 g)	F1	F2	F3	F4	F5	F6	F7	F8	F9	STD
Energy (kcal)	206 (2)	219 (4)	172 (0)	171 (0)	235 (4)	235 (4)	192 (0)	201 (2)	203 (2)	354
Protein (g)	14.45 (2)	13.74 (0)	13.58 (0)	13.17 (0)	15.94 (4)	15.54 (4)	15.15 (4)	14.62 (2)	14.56 (2)	12.00
Fat (g)	1.94 (1)	1.89 (0)	2.09 (2)	2.03 (2)	1.86 (0)	1.80 (0)	1.99 (2)	1.96 (1)	1.95 (1)	2.2
Carbohydrate (g)	63.42 (1)	64.27 (2)	65.37 (2)	65.94 (2)	60.69 (0)	61.26 (0)	62.61 (0)	63.24 (1)	63.31 (1)	65.3
Fiber (g)	9.65 (1)	9.52 (0)	9.31 (0)	9.00 (0)	10.32 (2)	10.01 (2)	9.77 (2)	9.67 (1)	9.66 (1)	12.2
Ash (g)	2.12 (1)	2.07 (0)	1.90 (0)	1.92 (0)	2.34 (2)	2.35 (2)	2.18 (2)	2.13 (1)	2.13 (1)	1.3
Ca (mg)	46.59 (1)	47.41 (2)	38.35 (0)	39.16 (0)	53.42 (2)	54.23 (2)	45.47 (0)	46.10 (1)	46.29 (1)	45.00
Fe (mg)	3.40 (2)	3.40 (2)	2.89 (0)	2.78 (0)	4.01 (4)	3.89 (4)	3.41 (4)	3.38 (0)	3.39 (2)	4.9
Mg (mg)	145.64 (1)	137.70 (0)	131.53 (0)	130.87 (0)	162.73 (2)	162.06 (2)	153.45 (2)	147.52 (1)	146.80 (1)	68
P (mg)	203.57 (1)	200.85 (0)	165.54 (0)	160.09 (0)	246.47 (2)	241.03 (2)	205.06 (2)	203.10 (1)	203.28 (1)	244
K (mg)	604.43 (1)	574.76 (0)	527.19 (0)	521.47 (0)	695.07 (2)	689.35 (2)	632.75 (2)	610.65 (1)	608.27 (1)	356
Na (mg)	9.80 (1)	9.27 (0)	7.81 (0)	7.73 (0)	11.99 (2)	11.91 (2)	10.29 (2)	9.89 (1)	9.86 (1)	5.00
Zn (mg)	2.60 (2)	2.46 (0)	2.56 (0)	2.49 (0)	2.76 (4)	2.69 (4)	2.75 (4)	2.64 (2)	2.63 (2)	2.00
Cu (mg)	0.52 (1)	0.49 (0)	0.51 (0)	0.50 (0)	0.56 (2)	0.55 (2)	0.56 (2)	0.53 (1)	0.53 (1)	0.27
Vit A (μg RE)	0.79 (2)	0.72 (0)	0.61 (0)	0.61 (0)	1.00 (4)	1.00 (4)	0.86 (4)	0.81 (2)	0.80 (2)	0
Vit E (mg)	1.07 (1)	0.99 (0)	1.22 (2)	1.21 (2)	0.97 (0)	0.96 (0)	1.17 (2)	1.10 (1)	1.09 (1)	0.23
Vit C (mg)	0.78 (1)	0.76 (0)	0.60 (0)	0.64 (0)	0.93 (2)	0.97 (2)	0.81 (2)	0.79 (1)	0.79 (1)	0
Thiamin (mg)	0.39 (1)	0.38 (0)	0.37 (0)	0.36 (0)	0.43 (2)	0.42 (2)	0.41 (2)	0.39 (1)	0.39 (1)	0.37
Riboflavin (mg)	0.12 (1)	0.12 (0)	0.12 (0)	0.12 (0)	0.13 (2)	0.13 (2)	0.13 (2)	0.12 (1)	0.12 (1)	0.09
Niacin (mg)	3.36 (1)	3.27 (0)	3.75 (2)	3.68 (2)	3.09 (0)	3.02 (0)	3.47 (2)	3.40 (1)	3.38 (1)	3.3
Pantothenic acid	1.05 (1)	0.95 (0)	1.34 (2)	1.34 (2)	0.81 (0)	0.81 (0)	1.16 (2)	1.09 (1)	1.07 (1)	n.a.
Vitamin B6 (mg)	0.41 (2)	0.43 (2)	0.40 (0)	0.41 (1)	0.40 (0)	0.41 (2)	0.39 (0)	0.40 (1)	0.41 (1)	0.49
Folate (μg)	191.83 (1)	180.60 (0)	153.66 (0)	155.99 (0)	230.31 (2)	232.65 (2)	202.32 (2)	193.97 (1)	193.15 (1)	40
Total score	29	12	10	11	44	46	46	26	28	-

RE, retinol equivalent; n.a., not available data.

- *Economic sustainability*

The prices ($/kg) of the raw flours (W, T, C and sorghum (S)) used for the creation of composite flours are shown in Table 4.

Table 4. Price ($/kg) of flatbread formulations based on raw material (W, T, S and C flours) costs in Sierra Leone, Tanzania, Burundi and Togo. W, wholewheat flour; T, tapioca; S, unsprouted sorghum flour; C, cowpea wholemeal flours.

Flour	Price ($/kg)			
	Sierra Leone	Tanzania	Burundi	Togo
W	0.71	1.38	0.79	1.17
T	0.59	1.08	0.31	1.10
S	0.66	1.29	0.68	0.50
C	0.71	2.15	0.73	1.26
F1	0.68	1.62	0.67	0.94
F2	0.68	1.58	0.66	0.96
F3	0.68	1.54	0.68	0.87
F4	0.67	1.53	0.66	0.86
F5	0.69	1.71	0.69	1.01
F6	0.68	1.70	0.67	1.01
F7	0.68	1.65	0.68	0.92
F8	0.68	1.63	0.67	0.94
F9	0.68	1.62	0.67	0.94

Since flour from sprouted sorghum is not commonly sold at the local markets but it is domestically produced by subjecting sorghum kernels to sprouting and milling, we reported the price of native sorghum flour, which is the type of flour commonly sold at the local markets. However, since sprouting represents a feasible and inexpensive technology traditionally performed at a domestic level in Africa (Abd Elmoneim, & Bernhardt, 2010; Singh et al., 2017), a similar cost is expected for the two types of flour. Among the 4 flours, for all the considered African countries, T and S were the ones with the lowest price. The price of T varied between a minimum of 0.31 $/kg (Burundi) to a maximum price of 1.10 $/kg (Togo); the price of S varied from a low of 0.66 $/kg (Sierra Leone) to a high of 1.29 $/kg (Tanzania).

In all countries, the cost of C was higher than that of T and S, varying from a minimum of 0.71 $/kg (Sierra Leone) to a maximum of 2.15 $/kg (Tanzania). Although cowpea is a widely cultivated and consumed legume in Africa (Jayathilake et al., 2018), the higher cost of the derived flour could be due to the fact that cowpeas are mainly consumed as a legume as it is, and not in the form of flour, which makes it a more difficult niche product to find on the market. The prices for W are also higher than for S and T, which is to be expected, since it is imported. W can be purchased for a minimum price of 0.71 $/kg (Sierra Leone) up to a maximum of 1.38 $/kg in Tanzania.

The costs of the formulations are a reflection of the percentage of each ingredient present in the blend and their prices in a specific country. The formulations proposed by the experimental design may have an average price of 0.68 $ /kg (Sierra Leone), 1.62 $/kg (Tanzania), 0.67 $/kg (Burundi) and 0.93 $/kg (Togo), and with the lowest price for all States, F4 was the least expensive composite formulation, thus meeting the economic sustainability criterion. Interestingly, all composite formulations resulted less expensive than STD (100% W) in Sierra Leone, Burundi and Togo, while the opposite resulted for Tanzania.

3.3 Characterization of selected flatbreads

3.3.1 Colour analysis

Colour parameters (L^*, a^* and b^*) of selected flatbreads and of STD sample are presented in Table 5. STD recorded the highest lightness ($L^*\approx67$) and the lowest redness ($a^*\approx4$) compared to composite flour-based flatbreads, while its yellow hue ($b^*\approx11$) was low but not significantly different from F4 ($b^*\approx10.6$). F4 in turn has the lowest brightness ($L^*\approx53$), while its red colour component was not significantly different from the other flatbreads ($a^*\approx6.5$), which may be due to SS strong reddish-brown notes and C lighter reddish notes. Among flatbreads, F6 had lighter coloured notes ($L^* \approx57$), probably due to the lower presence of SS in the blend, and more intense yellow notes ($b^*\approx14.6$), while F7 had intermediate parameters ($L^*\approx54.6$ and $b^*\approx13.1$). Overall, lower brightness and more accentuated yellow – red hues for blended flour flatbreads compared to STD may be attributed to the SS and C flours and possibly to a more extensive Maillard reaction occurring during cooking.

The ΔE values estimate whether colour differences between a sample made from blended flour and STD were perceivable to the human eye (the higher the value, the higher the perceived differences between the samples) (Limbo & Piergiovanni, 2006). ΔE value of 11.2 was found for the F6 sample, leading to a strong colour difference from STD, while ΔE values >12 revealed that the F4 and F7 samples were perceived as having a different colour from the STD (Fig. S1).

3.3.2 Sensory analysis

The consumer panel was composed of 15 men (56%) and 12 women, aged between 18 and 45 years (37% aged ≤25 years; 44% aged between 26 and 35 years and 19% aged ≥36 years) and coming from Ghana (6), Cameroon (5), Morocco (5), Togo (3), Gabon (3), Tanzania (1), Rwanda (1), Tunisia (1), Gambia (1) and Congo (1). As stated in the Material and Methods section, the number of judges were limited, due to difficulties for the consumers recruitment in the November 2020 lockdown period due to the COVID-19 emergency. Nevertheless, given the great relevance of the assessment of sensory properties of these flatbreads, some indications may be considered as worth of interest for further studies.

The average scores for flatbreads are presented in Table 5. Based on one-way ANOVA, significant differences ($p \leq 0.05$) were observed among the formulations both for acceptability and JAR test for all the measured sensory attributes [overall acceptability, consistency, appearance and flavour (acceptability test); hardness, darkness, bitterness and bean flour (JAR test)]. The scores recorded in the acceptability test for all the attributes revealed that the different kinds of flatbread were generally appreciated by the assessors (scores > 5). The consistency of the STD, F6 and F7 samples was evaluated in a similar way by the consumers (average score = 6.3), while the consistency of F4 obtained a significantly lower score (5.2). Those results were in agreement with textural properties measured analytically (Table 2), especially for the similar textural perception recorded for F6 and STD, and the worsen textural properties measured for F4 compared to STD.

With regard to appearance, STD was the most appreciated (7), followed by F6 (6.5) and F7 (6.4), and also for this attribute F4 obtained the lowest score (5.2). Flavour results showed the highest scores both for STD and F7 samples (6.9 and 6.8, respectively), followed by F4 (6.2) and F6 (5.4). Overall acceptability results revealed STD was the most appreciated sample (7.2), followed by F7 (6.5) and both by F4 and F6 (5.7 and 6, respectively), mainly due to their consistency and appearance (F4) or flavour (F6).

As for the JAR results, it was observed that the range of scores assigned by consumers was narrow around the JAR level (from a minimum score of 2.2 to a maximum of 3.9), and that therefore none of them had been evaluated overall at an extreme level, i.e., “much too low” or “far too much”.

Regarding the flatbread hardness, no significant differences were highlighted between STD, F6 and F7 samples (score = 3.2, 3.1 and 3.4, respectively), overall close to the JAR level, while the F4 sample obtained a significantly ($p \leq 0.05$) lower score (2.2), close to the “too low” level. Similarly, concerning darkness, no significant differences were found between the F4, F6 and F7 samples (3.9, 3.4 and 3.7, respectively), although the former came closer to the “too much” level, while W obtained an overall lower score (2.9), but close to the JAR level. As for bitterness, F6 received the highest score (3.7), followed by both F4 and F7 (3.2), and finally by W (2.7). The bean flavour of sample F6 obtained an evaluation close to the “too much” level (3.7), which may be expected given the highest percentage of C included in F6 formulation compared to others, while the others were closer to the JAR level and not significantly different from one another (2.8, 3.0 and 3.2 for STD, F4 and F6, respectively). Penalty Analysis (PA) was calculated to pinpoint potential roads to product improvement. The PA principle is that the highest score obtained in overall liking matches the JAR level; thus, for the PA the data obtained in the JAR test were used along with the overall liking scores of the acceptability test. The Spearman’s correlation coefficient demonstrated that the JAR variables did not affect significantly ($p \leq 0.05$) the overall liking scores obtained for the samples (data not shown). After aggregating the JAR scores obtained for the attributes of each sample (Fig. S2a) into a three-level scale (Fig. S2b), PA was performed to underscore how many points had been lost due to a product dimension judged “too much” or “too little” by consumers (Zhi et al., 2016). Table S1 shows a representative PA result (F4 bread), where the frequency of responses for the 3-level scale for each attribute, the sum of the liking scores corresponding to each level, and the average liking for each level were provided. Thus, the mean decreases for the “too much” and “too little” levels showed how many points of liking were lost for having a product that was “too much” or “too little” compared to the JAR level, while the penalty column shows how many points of liking were lost for not being as expected by the consumer. The p-value (significance level = 0.05) corresponded to the comparison test of the mean for the JAR level with the mean of the other levels and tested whether the penalty was significantly different from 0 or not. Overall, for all the dimensions analysed for all flatbreads, the test result was not significant, indicating that for those attributes, evaluated at a non-JAR level by the panelists, the product was not significantly penalized.

No significant differences (*p* ≤0.05) were found among the preference ranking data of the tested flatbreads collected in blind conditions. After an explanation of the project and samples, Friedman Nonparametric test attested significant differences among the preference ranking data (*p* ≤0.05), while multiple pairwise comparisons (Nemenyi procedure) revealed that the sample meeting the nutritional criteria (F7) became the preferred flatbread, followed by F6, F4 and STD (Table S2), which was the least preferred despite the high scores obtained in the acceptability test, possibly due to its perceived lower sustainability and nutritional value.

3.3.3 Relevant starch nutritional fractions

Relevant nutritional starch fractions were identified on the basis of the rate of glucose released and its absorption by the gastrointestinal tract during digestion. These include rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), defined as the three sequential nutritional starch fractions determined by reaction time when an *in vitro* starch digestion is performed (Collar, Jiménez, Conte, & Fadda, 2014).

RDS is the starch portion digested in the mouth and small intestine causing a rapid rise in blood glucose concentration after consumption of carbohydrates. The RDS fraction determined *in vitro* identifies the amount of starch digested within 20 min of a standard digestion reaction mixture (Englyst Kingman, & Cummings, 1992). Results for RDS were fairly close to each other and all significantly (*p* ≤0.05) lower in the blended flour flatbread samples (≈37 g/100 g d.b.) than in STD (≈48 g/100 g d.b.). SDS represents the starch portion digested slowly but completely in the human small intestine after RDS and identifies the starch portion digested in more than 120 min under *in vitro* standard conditions of substrate and enzyme concentration (Englyst et al., 1992). SDS offers potential health benefits, e.g., stable glucose metabolism, control of diabetes, mental performance and feelings of satiety (Lehmann & Robin, 2007). Results for SDS in composite flour bread ranged from ≈28 to ≈23 g/100 g d.b., in all cases higher than STD which recorded the lowest amount of SDS (≈17 g/100 g d.b.). Focusing on the % of RDS and SDS expressed on DS, i.e., the starch fraction available for digestion within 120 min (Englyst et al., 1992), F7 showed the lowest RDS percentage (and consequently the highest SDS percentage), followed by F6 and F4, which presented intermediate results, and STD, most of whose starch resulted rapidly digested (73%).

RS represents the starch portion escaping digestion in the small intestine (Englyst et al., 1992) which may undergo bacterial fermentation in the colon. Composite flour flatbreads contained amounts of RS ranging from ≈0.93 (F4) to ≈0.69 (F6) g/100 g d.b., which resulted, in all cases, significantly higher than the RS amount measured in STD bread (0.48 g/100 g d.b.).

Overall, all composite flour flatbreads recorded lower RDS and higher RS values than STD, which are thus considered appropriate nutritional trends for dietary starch fractions (Englyst et al., 2003). Since T is a source of readily available carbohydrates (Abass et al., 2018), and sprouting has been proven to increase starch digestibility (Gamel, Linssen, Mesallem, Damir, & Shekib, 2005; Lemmens et al., 2019; Section B, Chapter 1), the results observed for blended flour flatbread may be principally attributed to C. Incorporation of wholemeal legume flour in bread recipes appears to decrease starch hydrolysis, possibly owing to lower starch content but greater fibre and protein content. In fact, the reduced rates as well as an overall diminished starch digestibility of legumes are influenced by cell-wall structure, phenolic compounds, high amylose content and viscous soluble dietary fibre components. Additionally, the high protein content of legumes can promote starch–protein interactions which limit enzyme attack (Chung, Liu, Hoover, Warkentin, & Vandenberg, 2008; Angioloni, & Collar, 2012).

3.3.4 Total phenolic content (TPC) and DPPH• free radical scavenging activity analysis

The TPC of flatbreads is reported in Table 5. F7 showed the highest TPC (≈835 mg GAE/kg d.b), followed by F4 (≈771 mg GAE/kg d.b) and F6 (≈655 mg GAE/kg d.b), the latter resulting not significantly different ($p \leq 0.05$) from STD (≈651 mg GAE/kg d.b). The highest TPC measured for F7 flatbread may be related to the highest presence of SS and C in the used composite flour, which possibly enriched the finished product with bioactive compounds with potential beneficial effects on human health. Indeed, as known from the literature, like other legume flours, cowpea flour is a good source of phenolic compounds (Cai, Hettiarachchy, & Jalaluddin, 2003; Jayathilake et al., 2018). In addition, the positive function of sorghum sprouting on the phenolic content of wholemeal flour has been documented in detail in the literature (Gan et al., 2017; Lemmens et al., 2019; Salazar-López et al., 2018; Donkor et al., 2012; Section B, Chapter 2). Previous investigations have proved SS to have an increased total polyphenol content along with a rise in their soluble and bio-accessible fractions compared to flour made from unsprouted sorghum (Section B, Chapter 2), which make this flour an ingredient potentially able to increase the nutritional profile of finished products. It is scientifically proven that the species of reactive oxygen and the free radicals generated in cellular metabolism or the peroxidation of lipids and other biological molecules, play important roles in chronic diseases like coronary heart disease and also cancer. Antioxidants in the diet fight against free radicals, and probably help to prevent *chronic disease risk* factors (Ragaee, Guzar, Dhull, & Seetharaman, 2011). In this work, the antioxidant capacity of the bread samples was determined based on the DPPH• free radical scavenging activity and expressed as Trolox Equivalent Antioxidant Capacity (TEAC); the results are reported in Table 5. The TEAC values were 1.09, 1.03, 1.96 and 0.92 μmol Trolox eq./g d.b. for F7, F4, F6 and STD, respectively, with the last two not significantly differing from each other ($p \leq 0.05$).

As expected, Pearson analysis revealed a strong positive correlation between antiradical activity and TPC ($r=0.90$; $p < 0.05$), showing that the use of wholemeal SS- and C-enriched composite flours could deliver a source of natural antioxidant complexes with possible powerful and beneficial effects on health. Moreover, the improved TEAC observed in the composite flour bread samples could be additionally due to the documented rise in antioxidant vitamins after sorghum sprouting (Section B, Chapter 2), along with the antioxidant potential of cowpea proteins, mainly related to the capacity of hydrophobic and aromatic amino acids to donate protons to free radicals (Jayathilake et al., 2018), and to potential Maillard reaction products formed during baking (Michalska, Amigo-Benavent, Zielinski, & del Castillo, 2008).

Table 5. Colour, sensory and nutritional properties of flatbreads. In brackets, the percentage of RDS or SDS calculated on DS.

	F4	F6	F7	STD
Colour [a]				
L^*	53.3 ± 1.71d	57.11 ± 1.97b	54.63 ± 1.26c	67.37 ± 1.87a
a^*	6.38 ± 0.62a	6.58 ± 0.93a	6.89 ± 0.68a	3.8 ± 0.54b
b^*	10.59 ± 1.24c	14.62 ± 1.71a	13.15 ± 1.17b	10.94 ± 0.84c
ΔE	14.3	11.2	13.3	-
Sensory analysis [d]				
Acceptability test				
Consistency	5.22 ± 0.97b	6.26 ± 1.06a	6.15 ± 0.91a	6.33 ± 0.92a
Appearance	5.89 ± 1.01c	6.48 ± 1.09ab	6.44 ± 0.89b	7 ± 0.83a
Flavour	6.15 ± 1.03b	5.44 ± 0.97c	6.78 ± 0.97a	6.89 ± 0.89a
Overall acceptability	5.7 ± 0.99c	5.96 ± 1.09c	6.48 ± 0.94b	7.19 ± 0.68a
JAR test				
Hardness	2.19 ± 0.83b	3.07 ± 1.04a	3.41 ± 1.08a	3.22 ± 0.97a
Darkness	3.89 ± 0.93a	3.41 ± 0.8a	3.7 ± 1.03a	2.89 ± 0.97b
Bitterness	3.22 ± 1.01b	3.67 ± 0.68a	3.15 ± 0.99b	2.67 ± 0.62c
Bean flavour	3.04 ± 1.09b	3.7 ± 0.78a	3.15 ± 0.95b	2.81 ± 0.4b
Nutritional fractions of starch (g/100 g d.b.) [a]				
RDS	35.51 ± 2.26b (61)	37.56 ± 1.68b (62)	36.96 ± 0.79b (57)	47.55 ± 3.43a (73)
SDS	23.07 ± 1.11ab (39)	22.82 ± 2.66ab (38)	27.87 ± 1.41a (43)	17.3 ± 5.62b (27)
DS	58.58	60.38	64.83	64.85
RS	0.93 ± 0.01a	0.69 ± 0.04c	0.79 ± 0.08b	0.48 ± 0.02d
TS	60	61	66	65
TPC (mg GAE/kg d.b.) [c]	770.78 ± 0.69b	654.78 ± 1.25c	835.45 ± 1.40a	650.65 ± 1.20c
TEAC (μmol Trolox eq./g d.b.) [c]	1.03 ± 0.02b	0.96 ± 0.03c	1.09 ± 0.03a	0.92 ± 0.03c

[a] n ≥ 10; [b] n = 6; [c] n = 4; [d] n = 27. Values followed by different letters in each raw are significantly different (one-way ANOVA with Duncan's *post-hoc* test. $p \leq 0.05$). F4, formulation chosen on the basis of the economic sustainability criteria; F6, formulation chosen on the basis of the textural properties criteria; F7, formulation chosen on the basis of the nutritional criteria; JAR, Just-About-Right test; RDS, rapidly digestible starch; SDS, slowly digestible starch; DS, digestible starch (RDS+SDS); RS, resistant starch; TS, total starch (DS+RS); TPC, total polyphenol content; GAE, gallic acid equivalent; TEAC, Trolox Equivalent Antioxidant Capacity.

4. Conclusions

This work was aimed at developing nutritionally enhanced flatbreads for African countries by using wheat, tapioca, sprouted sorghum and cowpea flour blends, while preserving sensory acceptability, textural attributes and economic sustainability. Among those proposed, three flatbread prototypes were chosen on the basis of textural, nutritional and economic criteria, and further characterized for their physicochemical properties, sensory acceptability, *in vitro* starch digestibility, total phenolic content, and total antioxidant capacity in comparison to a reference 100% wholewheat flatbread (STD). Overall, if the goal is to produce a flatbread with a physico-chemical profile similar to the 100% W reference, the use of a composite flour made of SS, T, C and W at a percentage of 29.2, 12.5, 49.7 and 8.6 w/w f.b. respectively (F6) should be preferred. This formulation, in addition to being less expensive than a 100% W in Sierra Leone, Burundi and Togo, offers the highest content in energy and macronutrients and represents a good source of micronutrients. Moreover, it has similar TPC and antioxidant activity compared to STD.

Besides, if the objective is to meet the nutritional criterion, a flour blend made by SS, T, C and W at a level of 42.1, 7.4, 43.1 and 7.4% w/w f.b. respectively (F7) should be chosen. Other than having a delayed starch digestibility than the 100% W reference, it results having the highest score for micronutrients and a high rate for energy and macronutrients. In addition, it seemed to provide a higher content of phenolic compounds and antioxidant activity compared to the STD flatbread. Although the derived flatbread textural properties were found to be different from the STD when measured analytically, it appears that consumers did not detect significant differences in texture compared to the control, and even the flavour of this flatbread was perceived as acceptable as STD. However, consumers sensory perceptions on flatbreads should be confirmed with a larger panel.

Finally, if the primary objective is the economic sustainability, a composite made by SS, T, C and W at a percentage of 48.6, 12.5, 30.3 and 8.6% w/w f.b. respectively (F4), should be preferred to others. This formulation was proved to have a lower starch digestibility, a higher content of phenolic compounds and antioxidant activity than STD but worsen textural properties than the control sample.

Overall, the partial substitution of wheat flour with tapioca, sprouted sorghum and cowpea flours blended in different proportions resulted in some very interesting products which were not only perceived overall as acceptable by consumers, but also featured delayed starch hydrolysis and a higher total phenolic content and antioxidant activity compared to the wheat flour reference. Therefore, the use of composite flour in breadmaking was proved to be an efficient strategy to promote the use of locally available raw materials to obtain economically sustainable, nutritionally enhanced flatbread with an acceptable technological profile.

Supplementary Materials

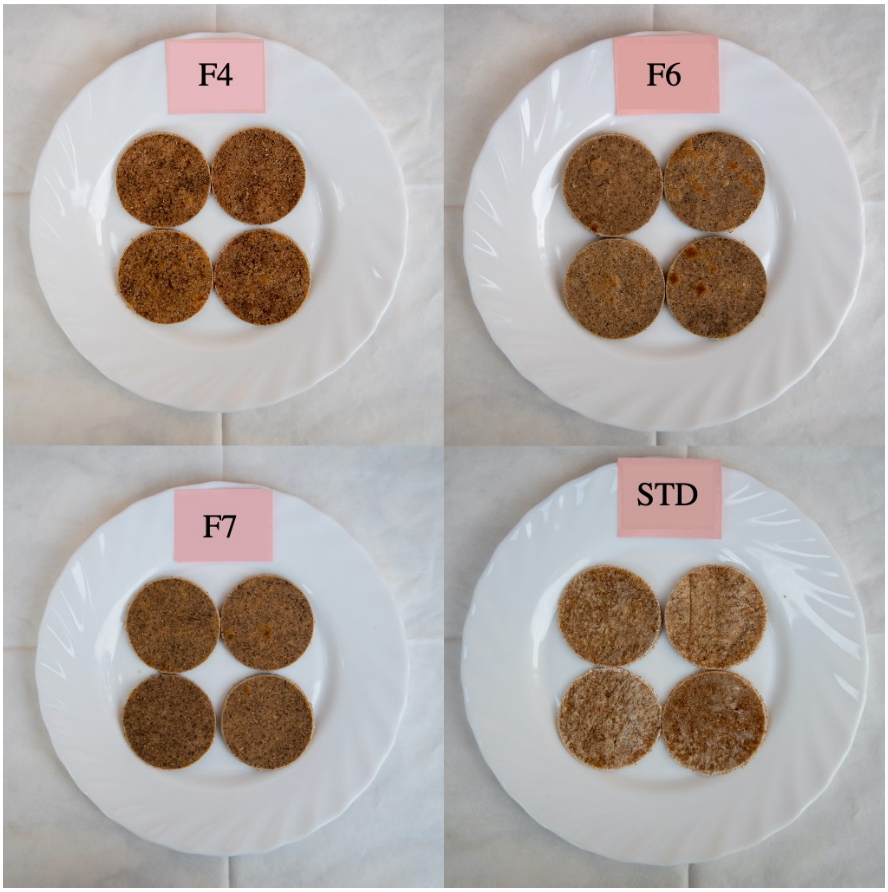

Fig. S1. Selected composite flour flatbreads on the basis of technological (F6), nutritional (F6 and F7) and economic sustainability criteria (F4). STD, standard 100% W bread.

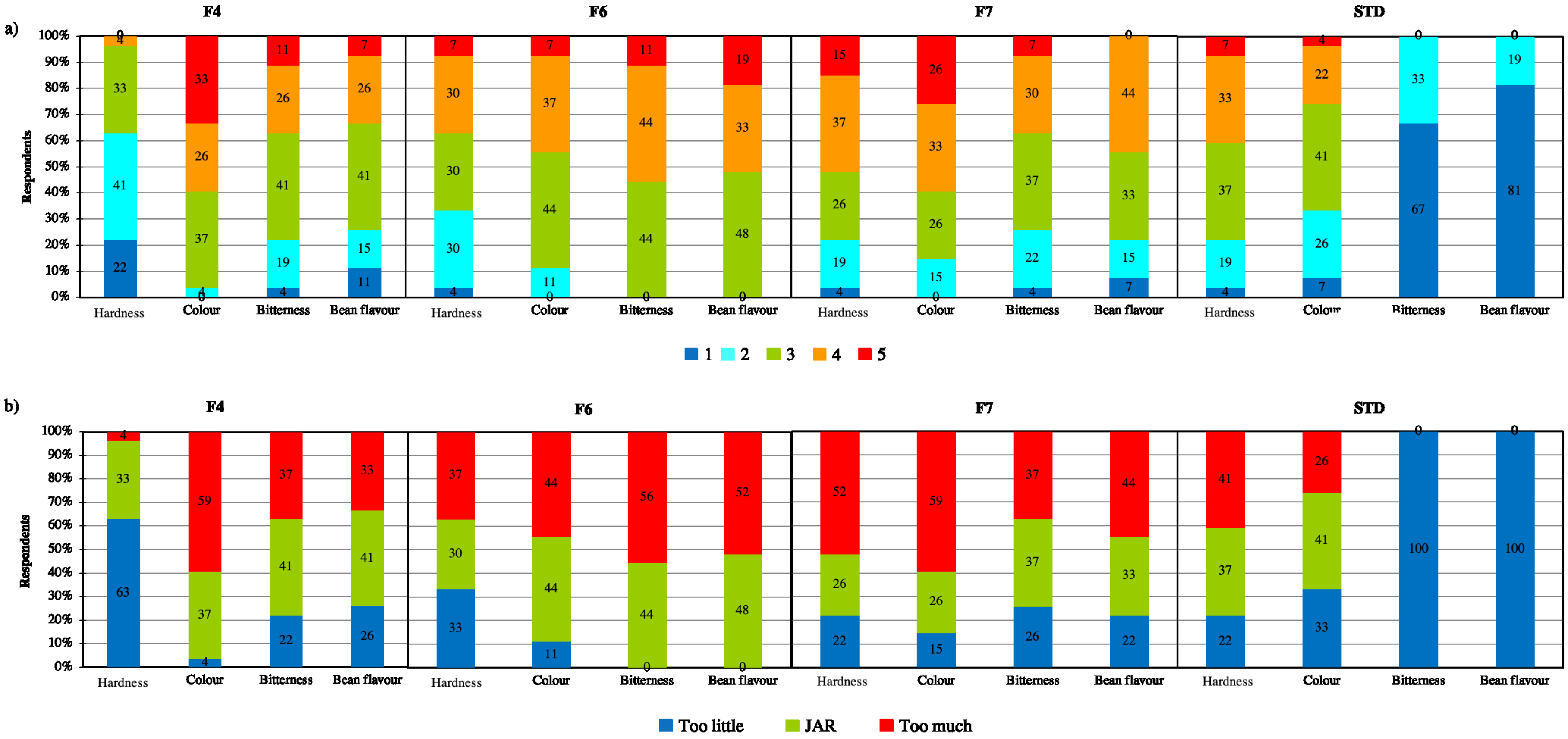

Fig. S2. Percentage of respondents (n=27) giving consumer ratings for breads' hardness, darkness, bitterness and bean flavour a) based on 5-point JAR scale; b) based on the collapsed on a 3-level scale.

Table S1. Penalty analysis table for F4 bread.

Variable	Level	Frequencies (%)	Sum (Overall liking)	Mean (Overall liking)	Mean drops	Penalties	*p*-value
Hardness	Not enough	62.96	96.00	5.65	0.13	0.11	0.790
	JAR	33.33	52.00	5.78			
	Too much	3.70	6.00	6.00	-0.22		
Darkness	Not enough	3.70	6.00	6.00	-0.40	-0.17	0.686
	JAR	37.04	56.00	5.60			
	Too much	59.26	92.00	5.75	-0.15		
Bitterness	Not enough	22.22	33.00	5.50	0.50	0.50	0.204
	JAR	40.74	66.00	6.00			
	Too much	37.04	55.00	5.50	0.50		
Bean flavour	Not enough	25.93	41.00	5.86	0.05	0.34	0.383
	JAR	40.74	65.00	5.91			
	Too much	33.33	48.00	5.33	0.58		

Table S5. Multiple pairwise comparisons (Nemenyi's procedure) performed on second preference ranking test data.

Sample	Rank Sum of Sample*	Rank Mean of Sample
F7	39c	1.44
F6	63bc	2.33
F4	67b	2.48
STD	101a	3.74

*Rank sum of the sample = $\sum$(number of panelists × respective rank position).
Lower rank sum indicated better-liked samples.

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Concluding remarks and future perspectives

This research, carried out with a multidisciplinary approach, focused on attempts to improve the use of food resources in African countries. In this regard, the development of high nutritional value food products with country-specific ingredients and through the optimization of locally feasible technologies were intended to bring value to African communities by strengthening local food systems' resilience to shocks while ensuring the right to food.

The attempts were specifically directed to the development of sustainable Ready-To-Use Therapeutic Foods (RUTFs) against child wasting, to the investigation of sprouting and drying treatment on sorghum as a potential way to modulate the flour's nutritional profile and functionality and to its use in blends with locally available raw materials to produce high nutritional and sustainable flatbreads.

In the first section of the research, an integrated multidisciplinary approach, which we called "Pappa di Parma", was adopted to develop, characterize, and introduce alternative energy-dense meals to tackle malnutrition which were, importantly, sustainable. Six formulations were developed using basic, easily accessible technologies and ingredients sourceable locally in Tanzania, a daily portion of which met the macronutrient requirements established for a RUTF and most micronutrients RNI (recommended nutrient intake). The *formulae*'s quality, rheological properties and shelf-stability assessed under different storage conditions revealed the suitability and stability of *no-water formulae* in all the storage conditions tested for at least three months. Moreover, we proved the cultural acceptance and economic sustainability of these *formulae* at local level. Overall, this study confirmed the "Pappa di Parma" approach as a valid starting point in developing sustainable bespoke alternatives to RUTFs in specific agricultural and socioeconomic contexts.

Besides, the review of the scientific literature dealing with the development of sustainable novel RUTFs, including acceptability evaluations, revealed that studies in the literature have rarely developed such novel RUTFs for a specific socioeconomic environment while also verifying cultural appropriateness and local acceptance. Moreover, for the most part, these RUTFs were unsustainable owing to the use of costly, difficult-to-source industrial ingredients, or production processes that were not scalable at a local level.

In addition, the need for community engagement and educational intervention in novel RUTF acceptance among children and their families was proved.

Accordingly, future research should apply a multidisciplinary, multi-stakeholder partnership approach to develop "fit-for-the-purpose" RUTFs, in order to answer families' expectations in terms of taste and cultural appropriateness and acceptability, value, convenience, ease of production and safety, while meeting nutritional requirements. Moreover, since meeting the micronutrient requirements established for RUTFs by making exclusive use of sustainable plant-based ingredients remains a challenge today, especially for micronutrients supplied by animal-based ingredients (e.g., vitamin B12), technological processes such as fermentation, traditionally performed on a domestic scale in low-income countries, or the use of fishery products should be urgently investigated to improve the nutritional value of plant-based ingredients.

The second part of the research studied the effects of sorghum sprouting and different drying treatments (40 °C for 12 h vs. 50 °C) on the nutritional and technological features of derived flours to be used to develop bakery products targeted to African countries.

With regard to the protein and starch profiles, we found an increase in total amino acid content in the flour samples upon sprouting, an increase in water and oil holding capacity (WHC and OHC) but a worsening in starch gelatinization properties, thus confirming, as expected, that sprouting affected the functionalities of the protein and starch in the flour. However, significant differences were found in the effects of the drying treatments adopted: drying performed at a lower temperature/longer time (40 °C for 12 h) was proved to extend the enzyme activity triggered by the sprouting than drying at a higher temperature/shorter time. Increased protein hydrolysis, WHC and OHC were found in the flour obtained using the former treatment. The higher protein matrix hydrolysis resulted in a high exposure of starch granules to the enzymes, increasing digestibility but worsening technological functionality. Additionally, the sprouting processes applied to sorghum afforded a sustainable strategy to improve the micronutrient profile of derived flours, with a decrease in phytic acid, an increase in vitamin, mineral and total polyphenol content along with their soluble and bio-accessible fractions, and a positive impact on the antioxidant potential of sorghum flour. In particular, the combination of a lower temperature (40 °C) and longer time (12 h) in drying resulted in lower *thermal degradation of the vitamins* and extended metabolic and enzymatic pathways. The latter are involved in soluble phenolic compound biosynthesis and transformation which are boosted by the sprouting process, and thereby increase the soluble bio-accessible polyphenols and the flour's free radical scavenging activity.

Based on the above, modulating drying conditions after sprouting appeared to be an effective, sustainable method to improve the nutritional profile of sorghum flour, and therefore deserves to be further deepened.

In the study of the rheological (by means of Dynamic Mechanical Analysis) and molecular (by means of Low-Resolution ^{1}H NMR technique) properties of doughs from spouted sorghum flour, it was highlighted the effect of sprouting on biopolymers-biopolymers and biopolymers-water molecular interactions. Interestingly, these differences were not reflected at rheological level. The poor viscoelastic properties of sorghum were therefore not further worsened as a result of sprouting and this was evaluated of particular interest. Indeed, as the use of sorghum flours find application where high viscoelastic performance and/or intensive starch gelatinization is not requested, these results bolster the use of sprouted sorghum flour rather than the unsprouted one, being the former characterized by a significantly higher nutritional value than the latter.

Taking all the aforementioned results into account, the flour obtained from the grain which underwent drying at 40 °C for 12 h was earmarked as a sustainable food ingredient which could potentially improve the nutritional profile of finished bakery products. In the subsequent study, this flour was used in blend with tapioca, cowpea and wholewheat flours to produce composites with potential high nutritional value, which functional properties were studied. Results showed that the composite flour made up of sprouted sorghum flour, tapioca, cowpea flour and wheat flour at 25% w/w flour basis could represent a sound compromise between the flour's technological and nutritional quality, thus bolstering the use of this flour in the development of bakery products targeted to African countries, while helping to reduce the use of imported wheat flour as well as cutting cassava post-harvest losses.

Accordingly, the formulation was optimized in order to develop nutritionally enhanced, economic sustainable and textural-quality acceptable flatbread targeted to African countries.

Three flatbread prototypes within the experimental set defined by the experimental design were chosen for their superior textural, nutritional, and economic features, and further characterized for their physico-chemical properties, some nutritional traits and sensory features. Overall, the partial substitution of wheat flour with sprouted sorghum, tapioca, and cowpea flours blended in different proportions resulted in some very interesting products which featured delayed starch hydrolysis and a higher total phenolic content and antioxidant activity, acceptable textural quality and high sustainability compared to the reference 100% wholewheat flatbread. In view of this, research on the potential effects of these products on the health of consumers as well as a deepen assessment about their cultural and sensory acceptability could be of interest for the future.

Overall, optimizing the sprouting process along with post-sprouting drying was proved to be a sustainable, locally feasible method to improve the nutritional profile of sorghum flour. Additionally, the use of sprouted sorghum flour in composites represents an efficient strategy to promote the use of locally available raw materials to develop technologically satisfactory, highly nutritional, and economically sustainable bakery products to use in the daily diet.

For this research to provide a concrete, sustainable and effective method to local communities to build food security and resilience, these products should be implemented in Africa in order to verify their feasibility in local contexts. In addition, a specific education program should also be implemented in order to transfer knowledge and skills to local people, while involving local institutions and governments to be more effective.

In conclusion, the outcomes of this Ph.D. research provide useful and scientifical based - information usable to different stakeholders with the ultimate goal to help food-insecure African communities to build resilience and acquire sufficient, safe, nutritious food in the long-term with greater reliance on local agriculture and less dependency on food aid.

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The Author

Mia Marchini was born on August 15th, 1992 in Cremona, a province of the Lombardy region, Italy.

After obtaining her High School Leaving Certificate (Specializing in Classical studies), she enrolled in the Bachelor's course in Food Science and Technology at the University of Parma. She obtained her Bachelor's Degree with top marks of 110/110, defending an experimental thesis entitled: "Microbiological analysis of vegan and lacto-ovo-vegetarian foods. Comparison between commercial and home-made foods".

Mia continued her studies by enrolling in the Master's course in Food Science and Technology, again at the University of Parma. In January 2017 she graduated with top marks of 110/110 *cum laude*, defending a thesis entitled: "Rheological characterization of semolina for bread with different analytical techniques: comparison between traditional and alternative methods". The research work was carried out at Barilla G. e R. Fratelli S.p.A. In November of the same year, she passed the selection process to attend the University of Parma's Ph.D. Course in Food Science, under the supervision of Professor Eleonora Carini.

During the Ph.D. research period, Mia enjoyed an unforgettable experience in Tanzania, at the Mvimwa Abbey (Rukwa Region), where she had the opportunity to implement the "Pappa di Parma" project and become familiar with the local state of affairs.

During the Ph.D. research period, she investigated various research topics, such as the use of lentil flour in breadmaking and the procedure of Low-Resolution Proton Nuclear Magnetic Resonance (LR ^{1}H NMR) to describe the step of kneading wholewheat dough.

From November 2017 to October 2018, she worked as a tutor for the students on the Bachelor's and Master's courses in Food Science and Technology.

Mia also held the role of Teaching Assistant for the course "Structure and Physicochemical Properties of Foods", a Master's course in Food Science and Technology (Professor Eleonora Carini) for the Academic Years 2018-2019 and 2019-2020.

Since 2017, she has been collaborating as a food technologist with Open Fields Srl, a company based in Parma committed to the agri-food industry, which provides services for innovation and technology transfer.

At Open Fields, Mia contributes to the planning, coordination and reporting activities of Regional and European Innovation Projects in the agro-industrial sector, to R&D consultancy for companies in the agri-food sector for the development of innovative products/processes and to corporate communications.

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Main Scientific Activities

Original Papers

- ***The "Pappa di Parma" integrated approach against moderate acute malnutrition.***
  Authors: Mia Marchini, Alice Rosi, Francesca Giopp, Francesca Scazzina, Eleonora Carini.
  Source: *Innovative Food Science & Emerging Technologies*. Volume 66, December 2020, 102534.
  DOI: https://doi.org/10.1016/j.ifset.2020.102534 *(published)*.

- ***Sprouting of sorghum (Sorghum bicolor [L.] Moench): effect of drying treatment on protein and starch features.***
  Authors: Mia Marchini, Alessandra Marti, Claudia Folli, Barbara Prandi, Tommaso Ganino, Paola Conte, Costantino Fadda, Monica Mattarozzi, Eleonora Carini.
  Source: *Foods,* 10(2), 407.
  DOI: https://doi.org/10.3390/foods10020407 *(published)*.

- ***The use of red lentil flour in bakery products: How do particle size and substitution level affect rheological properties of wheat bread dough?***
  Authors: Mia Marchini, Eleonora Carini, Nicolò Cataldi, Fatma Boukid, Massimo Blandino, Tommaso Ganino, Elena Vittadini, Nicoletta Pellegrini.
  Source: *LWT – Food Science and Technology.* Volume 136, Part 1, January 2021, 110299.
  DOI: https://doi.org/10.1016/j.lwt.2020.110299 (*published*).

- ***Use of the ^{1}H NMR technique to describe the kneading step of wholewheat dough: The effect of kneading time and total water content.***
  Authors: Ottavia Parenti, Lorenzo Guerrini, Bruno Zanoni, Mia Marchini, Maria Grazia Tuccio, Eleonora Carini.
  Source: *Food Chemistry*. Volume 338, 15 February 2021, 128120
  DOI: https://doi.org/10.1016/j.foodchem.2020.128120 (*published*).

- ***Wholewheat bread: effect of gradual water addition during kneading on w dough and bread properties***
  Authors: Ottavia Parenti, Eleonora Carini, Mia Marchini, Maria Grazia Tuccio, Lorenzo Guerrini, Bruno Zanoni
  Source: *LWT – Food Science and Technology.* Volume 142, May 2021, 111017.
  DOI: https://doi.org/10.1016/j.lwt.2021.111017 (*published*).

- ***Sustainability and acceptability of novel Ready-to-Use Therapeutic Foods in acute malnutrition management - A systematic review***
  Authors: Mia Marchini, Alice Rosi, Francesca Raia, Elena Bertolotti, Francesca Scazzina, Eleonora Carini.
  Source: *Trends in Food Science & Technology (submitted)*.

- ***Drying after sprouting as a potential way to improve the micronutrient content, in vitro bioavailability and antioxidant activity of sorghum flour (Sorghum bicolor* [L.] Moench*).***
  Authors: Mia Marchini, Paola Conte, Costantino Fadda, Eleonora Carini.
  Source: *Journal of the Science of Food and Agriculture* (*under review*).

- ***Insight into molecular and rheological properties of sprouted sorghum flour***
  Authors: Mia Marchini, Riccardo Arduini, Eleonora Carini.
  Source: *Food Chemistry (accepted).*

- ***Functional properties of sustainable composite flours from sorghum, tapioca and cowpea***
  Authors: Mia Marchini, Alessandra Marti, Maria Grazia Tuccio, Elena Bocchi, Eleonora Carini
  Source: *To be submitted.*

- ***Towards sustainable and nutritionally - enhanced flatbread targeted to African countries***
  Authors: Mia Marchini, Maria Paciulli, Lorenza Broccardo, Maria Grazia Tuccio, Francesca Scazzina, Martina Cirlini, Eleonora Carini
  Source: *To be submitted.*

Workshops and Schools

- Course “Tecnologia e merceologia molitoria. Processi, diagrammi, prodotti. Corso di formazione sulle tecniche e tecnologie innovative in campo molitorio. Organized by Italmopa, Antim, Cisita Parma, Unione Parmense degli Industriali. October-December 2020.
- International Summer School on Food Sustainability. University of Parma. On-line, 1 June – 31 July 2020.
- Training on Multivariate Data Analytics Solution – software SIMCA. S-IN soluzioni informatiche. 12-13 December 2019.
- Training on Experimental Design – software MODDE Pro. S-IN soluzioni informatiche, Dr. Lorenza Broccardo. 14-15 November 2019
- Parma Summer School 2019 “Risk-benefit in food safety and nutrition”. University of Parma and EFSA, 11-13 June 2019.
- 24th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology. Poster contribution: *Improvement of the use of food resources and reduction of post-harvest losses in African countries.* Firenze, Italy 11-13 September 2019.
- Training School: NMR relaxometry data analysis: theory and software. University of Pavia, Italy, 18-22 February 2019.
- School of Multivariate analysis - Italian Chemical Society - Analytical chemistry division. Department of Pharmacy, University of Genoa (DIFAR), Italy, 21-25 January 2019.
- 23rd Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology. Poster contribution: *Improvement of food resources use and reduction of post-harvest losses of local products of African countries*. Oristano, Italy, 19-21 September 2018.
- Rheology Seminar. Dr. Thomas Mezger Anton Paar Italia, Rivoli, Turin, Italy, 7-8 March 2018.