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Doctorate on **Management of innovation in the agricultural and food systems of the Mediterranean Region**

Development and valorisation of a nutritionally rich minor cereal (*Eragrostis tef*) by conventional and innovative techniques

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ABSTRACT

Teff (*Eragrostis tef*) is a minor cereal, which has attracting growing interest in the last years because of its high adaptability to harsh environmental conditions. It is consumed as a whole grain so it contains high amount of fibre and bioactive compounds as well as it presents an excellent nutrient profile due to a well-balanced aminoacid composition and high content of various minerals and vitamins. For all these reasons and due to the increasing global interest in healthy foods, teff has the potential to be used in products with improved functional properties.

The main aim of this research was to develop the knowledge for affirming the use of such minor cereal, *Eragrostis Tef*. So, it has been studied starting from its cultivation up to the product development.

The specific goals of this research were:

- The study of the adaptability of teff and other two minor cereals, sorghum and millet, to a Mediterranean area under deficit irrigation. Yield parameters, nutritional properties and antioxidant capacity were evaluated.
- The development of a healthy ready-to-eat cereal breakfast with the extrusion-cooking technology by using teff flour and by varying some extrusion variables, such as temperature of the last barrel zone and feed moisture of the blends. Specifically physical and structural properties, as well as pasting properties and some functional essential attributes (e.g. antioxidant capacity, phenolic content, fibre content) of extruded samples were investigated.
- The adoption of an emerging technology, 3D food printing, to obtain innovative and personalized snack, nutritionally designed to meet the specific micronutrient requirements of women. Also in this study teff flour was used in the food formulas.

The main results of this research study have permitted some considerations:

- Teff appears to be the best-adapted crop, among those studied, to the sub-arid environment of South of Italy due to its best yield stability and nutritional quality also showing a great potential to contribute to food and nutrition security in Mediterranean environments.
- The incorporation of teff flour significantly changed the physical and sensory characteristics of extruded cereals. In particular lightness and crispiness were affected

by inclusion of teff flour, but process management of extruded cereal breakfast, according to different process conditions, such as feed moisture and temperature of the last barrel, allows creating desirable quality of final products. Moreover, varying the process parameters and increasing teff flour addition, microstructural properties of sample change considerably and these characteristics could affect structural properties of extruded products.

- Teff flour served as a good source of polyphenols, which are the major contributors to the antioxidant capacity, in extruded ready-to-eat cereal breakfast. Under optimal conditions, the extrusion-cooking technology is a suitable method for the preparation of gluten-free products with high level of dietary fibre.
- Teff flour has been suitable for the 3D printing technology to obtain personalized foods. 3D printed snack met the micronutrient requirements of women, particularly iron, folate and calcium. These minerals/vitamins reached the RDA percentage requested to obtain the nutritional claim for such micronutrient. Moreover 3D printed snack showed a high fibre content so they also contribute to the women' health condition. Finally such snack were well appreciated by the panelists, assuming that consumers could accept this innovative and personalized products.

CHAPTER 1. Introduction

1.1 Minor cereals

Some cereal species, such as millet, sorghum and teff, are attracting growing interest in the last years (Wu et al., 2107). They are known as 'minor cereals' because of the lower cultivated land for their production respect to the major cultivated cereal crops, which are corn, wheat, rice and barely.

These cereal crops are largely cultivated in developing countries, where they represent a basic staple food of rural communities (Pontieri 2014; Proietti et al., 2015). In developed countries are generally used for animal feed, even if their consumption for humans have being explored recently (Shahidi & Chandrasekara, 2013; Afify et al., 2015; Queiroz 2015).

One of the reasons for this renewed interest is their excellent nutrient profile. In addition to being an important source of energy due to their starch content, these cereals provide high quality of protein, dietary fibre and lipids rich in unsaturated fats. Moreover, they contain adequate levels of important micronutrients such as minerals and vitamins as well as significant amount of bioactive compounds (Gerembramian, 2014; Ganatapathy et al., 2015). For all these reasons minor cereals are achieving resounding success in the market of healthy foods (Pradeep & Sreerama, 2017).

Minor cereals are known to be tolerant to extreme climatic and soil conditions resulting to be among the favourite crops in semi-arid areas with moisture limitations (Taylor et al., 2014). Moreover, under a climate change, annual mean precipitation is projected to decrease in many subtropical dry regions and mid-latitude (Wu et al., 2017). So they might be grown in these regions because of their adaptability to harsh environmental conditions and the minimum impact on yield and other quality parameters (Galindo et al., 2017; Queiroz 2015).

Furthermore, the absence of gluten makes them suited ingredients to meet the demands of gluten-free products (Inglett et al., 2016; Queriroz et al., 2015; Taylor et al., 2014).

Teff [*Eragrostis tef* (Zucc.) Trotter] belongs to grass family, traditionally harvested for grain in Ethiopia, where it is believed to have been domesticated (Haileselassie et al., 2016; Baye, 2014). Apart its adaptability to abiotic stress conditions it also works as an emergency or rescue crop after a crop failure and it does not require pest-controlling storage chemicals (Yihun et al., 2013; Norberg et al., 2009). In fact in Ethiopia, where more than half of the area under cereals is for teff production, farmers earn more from growing teff than growing other crops (Araya et al., 2010).

Teff is considered the smallest cereal of the world, taking 150 grains to weight as much as one grain of wheat (Haileselassie et al., 2016; Lacey & Carol, 2005). Because of this minuteness teff grains cannot be decorticated so it is consumed as a whole grain, containing high amount of bioactive compounds and fibre (Zhu, 2018). Its carbohydrate, protein and fat content is similar to other cereals but its excellent nutrient profile is a result of the well-balanced amino acid composition (Baye, 2014). In addition, it exhibits significant levels of minerals, including calcium, iron, magnesium, phosphorus, potassium and zinc, as well as of certain vitamins, such as thiamine, riboflavin, vitamin A and K (Gembremariam, 2014; Inglett et al., 2016).

It is an interesting alternative raw material for manufacturing nutritionally improved gluten-free foods, which, in general, not meet the recommended daily intake for fibre, minerals and vitamins. Additionally to the health benefits for celiac patients and to those related to the antioxidant activity of phytochemicals, it also contains some other compounds and high unsaturated fatty acids which exhibited anti-hyperlipedaemic and anti-hyperglycaemic activities as well as it is highly recommended for improving osteoporosis and bone healing (Inglett et al., 2016; Taha et al., 2012).

The principal use of teff grain for humans is 'Injera', a soft, porous, fermented flat bread (Yihun et al., 2013). Sometimes the flour is also used for making porridge, a unleavened bread named *kitta,* or alcoholic beverages (Gebremariam, 2014). Apart of these traditional foods, recently researchers are studying the potential use of teff in other food applications. Some studies focused on the development of teff-based products, like bread (Alaunyte et al., 2012; Marti et al., 2017), cookies (Inglett et al., 2016), muffin (Valcarcel et al., 2012), breakfast cereal (Robin et al., 2015; Solomon, 2014), pasta (Hager et al., 2012,2013; Giuberti et al., 2016), various sourdough (Wolter et al. 2014; Campo et al., 2016), fat replacer (Teklehaimanot et al., 2013), malt (Gebremariam 2015). Overall, teff flour addition increased the nutritional values of the products, but decreased the textural and sensorial quality.

To sum up, research opportunities exist to improve the status of teff as a sustainable crop and as a popular healthy food item, but further knowledge on its functional and physical properties needs to be implemented.

1.2 Breakfast Cereals

The obesity epidemic has resulted in a growing interest of food retailers and manufacturers to develop strategies aiming to reduce caloric intake and to improve dietary quality in the world. One dietary pattern that is promoted providing positive nutrition benefits is the consumption of breakfast cereals. In particular ready-to-eat (RTE) cereals is a prevalent food in the diet of people.

In the last years the market of breakfast cereals has been expanding in Italy, even if it is ever more developed in the United States and in the rest of the Europe (Sckokai & Varacca, 2012). Today this sector reach a high level of differentiation, in fact manufacturers introduced products with new flavours or fruits, convenient and on-the-go packaging, healthful multigrain ingredients, added fibre, etc. (Lee et al., 2007). This new products are frequently developed in order to exploit the healthy breakfast cereal market, which tends to enjoy higher profit margin compared to regular breakfast cereals. Recently food industry have introduced in the packaging of RTE cereal breakfast various claims on health and sensory benefits (Lee et al., 2007). In EU such claims are regulated in order to avoid misleading messages by producer and to inform precisely consumers about health benefits of products (Cavaliere et al., 2015). The specific nutritional content of food products expressed by nutritional claims (for example, 'low energy', 'sugar free', etc.) may be of interest to a specific typology of consumers particularly concerned with the nutritional aspects of their diet choices. Whereas, health properties of food products highlighted with health claims, could attract different consumers that are more interested in the direct link between food and health. These claims represent a simple and immediate tool, that can contribute to make consumer choices more aware and in line with individual preferences, favouring a higher transparency in the market.

Breakfast cereals (BC) were developed as the first "healthy food" as part of a whole grain diet (Clark, 2006). Flaked, puffed, shredded, and extruded RTE cereals are made from whole grains or parts of grains of corn, wheat, rice or oats. The role of BC in a balanced diet has been recognised for many years (Williams, 2014). The high density of BC, especially those produced with whole grain or with high fibre content, makes them an important source of key nutrients. Eating whole grain BC has been associated with lower risks of cardiovascular disease and total mortality, diabetes, hypertension, and weight gain in man, as well as it improves digestive health, dental and mental health and cognition (Williams, 2014). In particular, Ashwell et al., (2014), demonstrated that people who consumed high level of BC decreased their weight and body mass index. Another study highlighted that the prevalence and risk of overweight was lower in children and adolescent who consume breakfast cereal regularly compared to those who consume them infrequently (de la Hunty et al., 2013). Other studies have founded that eating breakfast cereals regularly is associated with better micronutrient intakes and healthier micronutrient profile (Gibson, 2003). However, not all breakfast meal types are associated with health benefits (Cho et al., 2003). In 2012 Harries et al. published a report documenting the nutritional quality of 261 ready-to-eat cereal breakfast offered by 12 companies in USA (Harries et al., 2012). They examined in particular nutritional content of cereals addressed for children and adolescents. Results of these study concluded that respect to a previous similar study it was observed a statistically significant reduction of sodium and sugar content, while fibre content didn't change significantly. However, according to nutritional analysis and to standards established by government agencies, none of the products investigated should be qualified as 'nutritious products' and should not be marketed, especially for children (Harries et al., 2012).

In summary, BC are relatively inexpensive, nutrient-dense, and convenient foods, which may be recommended to form part of a healthy balanced diet, but an accurate choice of the products should be conducted.

1.3 Conventional process: extrusion cooking technology

Extrusion technology has becoming more and more popular in the production of snack foods due to its technological advantages over the traditional food processing techniques (Ibanoglu et al., 2006). It combines multiple unit operations (e.g., mixing, blending, cooking, forming) in one single machine, which increases productivity and reduces production costs (Teba et al., 2017). The extruder function as a complex and complete processing unit, makes this technology capable to convert more than one kind of raw material into one fully cooked food product. Also, the process allows a precise control over the cooking parameters and process optimization (Berrios et al., 2013). For a hot extrusion process, high temperature, high pressure and short time duration are typical conditions to produce highly expanded and low dense products with unique texture properties (Stojceska et al., 2009). During this process, the raw materials undergo many chemical and structural transformations, such as starch gelatinization, protein denaturation, complex formation between amylose and lipids, as well as degradation reactions of vitamins, pigments and functional compounds (Teba et al., 2017; Peressini et al., 2015). The severity of functionality was affected by the process conditions and was aggravated by heating (Teba et al., 2017).

The nutrient density of extruded foods has been low, once these products have been predominantly made from rice or corn flour, with high levels of carbohydrates.

Successful incorporation of fruits, vegetables, gluten free cereals and whole grains into extruded products, that deliver physiological active components, could create a major opportunity for food processors to provide healthy dietary products (Stojceska et al., 2010; Potter et al., 2013; Oliveira et al., 2015).

Apart of the nutritional and functional properties, extrusion is always preferred over conventional cooking because of the distinct textural properties of the end products, i.e. high expansion ratio, low density, crispness, crunchiness, etc. All these parameters, which are significantly affected by the used ingredients and process parameters, are crucial in determining the acceptability of the products for consumers (Makila et al., 2014).

Therefore, extrusion cooking technology is identified as a useful process in respect of the development of a healthy snack well appreciated by consumers also for their structural characteristics.

1.4 Innovative technique: 3D food printing

3D printing (3DP) is an emerging technology that involves the layer-by-layer deposition of materials to form a 3D structure that may not have been achievable with conventional manufacturing techniques (Hamilton et al., 2018). It is also known as ‘additive manufacturing (AM) or rapid prototyping (RP)’ (Wang et al., 2018). This process begins with a 3D computer model created by acquiring image data or structures built in computer-aided design (CAD) software. Then a .stl (Surface Tessellation Language) file is created and the mesh data are sliced into a build file of 2D layers which is finally send to a 3D printing machine (Wang et al., 2018). In the last years, 3DP has opened a series of fascinating opportunities in several areas of research, the most interesting belonging to prototyping fabrication, engineering, material science, regenerative medicine, pharmaceutical, aerospace, etc. (Asharaf et al., 2018; Bose et al., 2013; De Obaldia et al., 2015; Goyanes et al., 2014; Hwa et al., 2017; Pati et al., 2015).

In food sector 3DP is currently of significant interest for numerous applications. Various ingredients are used to fabricate 3D printed food, like cheese (Le Tohic et al., 2018), potatoes (Liu et al., 2018), cereals (Lipton et al., 2015; Severini et al., 2016), edible insects (Severini et al., 2018a), fruits and vegetables (Yang et al., 2018; Severini et al., 2018b), chocolate (Hao et al., 2010), etc.

The main advantages of this technology are: mass customisation, geometric design freedom, low volume economy and less processing waste (Holland et al., 2018).

In particular the personalization of foods in its design, in terms of shape, dimension, colour, flavour, as well as in its nutritional value seems to be the best promising area of research. This is because consumer's attitudes for food decision are driven by five principal criteria: taste, cost, experience, convenience and nutrition (Deloitte et al., 2015). Specifically, food products that focus on personal care, healthy concepts and functional claims are emerging as a new trend due to consumers' growing attention to their health (Sun et al., 2018). Additionally it is well recognised that food ingredients and their effects on metabolism vary among individuals. This motivates a new market for personalised healthy food, which aims to tailor and fabricate products specifically designed on individual's health conditions. Traditional mass food preparation process cannot meet such personalized demands so there is a need for innovative methods of food processing to create these personalised foods. Under this scenario, 3DP could be a suitable technology to print edible materials into complex shapes and gives the users the ability to modify properties like texture, colour, and nutrition (Hamilton et al., 2018). For instance, researchers at the former Cornell Creative Machines Lab elaborated a system comparing the activity logged by a user in their Google calendar with their metabolic requirements and distributed 3D printed identical cookies with their caloric levels adjusted to the user's caloric deficit (Lipson & Lipton, 2013). Derossi et al. (2018) investigated the use of 3D printing technology to develop a personalised fruit-based food formula nutritionally designed to meet the micronutrient requirements for children. The EU performance project was launched with the goal to produce 3D printed smooth foods customised for people affected by mastication and swallowing problems, such as the elderly.

So, 3DP is a powerful technology, which shows capability to promote product innovation and functionality, but the available applications are still primitive and needs further investigations.

1.5 Objectives and outlines of the research

The main aim of this research was to develop the knowledge for affirming the use of a minor cereal, *Eragrostis Tef*. Due to the increasing global interest in healthy foods, teff have the potential to be used in products with improved functional properties. So it has been studied, starting from its cultivation up to the product development.

First of all in **chapter 2** a new simple and direct procedure for antioxidant activity assessment of food matrices, QUENCHER approach, was optimised for large solid particles of grounded wheat as a standard grain, because these large particles may have a physiological interest. This method avoids any extraction and hydrolysis step and it allows to overcome the problem of the lost of possible synergistic interactions among antioxidants.

The optimised procedure was then applied throughout all the research for studying the antioxidant capacity of grains and products developed with teff flour.

In **chapter 3** teff and other two minor cereal crops, millet and sorghum, were cultivated in a Mediterranean area to study their adaptability to this environment under deficit irrigation. The hypothesis of this study was that under water availability limited conditions, sorghum, millet and teff might be possible alternative crops in the studied area, where, in general, wheat and rice are largely cultivated. Agronomical and nutritional properties, as well as antioxidant capacity of these crops were investigated under full and limited irrigation.

Chapters 4 and **5** were dedicated to the introduction of teff flour into ready-to-eat breakfast cereals. More specifically, the effects of teff flour addition and some extrusion variables, feed moisture and last barrel temperature, were studied in order to improve our knowledge on the use of this cereal to obtain innovative and healthy breakfast products. Specifically, Chapter 4 focused on the effect of teff flour and extrusion process variables on some physical and structural properties, such as expansion ratio, colour, texture, because of their connection with the acceptability of consumers. In addition, a X-ray computed microtomography analysis was conducted in order to well understand structural changes occurred on extruded cereal breakfast. Chapter 5 attempts to affirm the functional properties of teff flour. In this case soluble and insoluble fraction of dietary fibre, phenolic content, total antioxidant activity, as well as pasting properties of the extruded samples have been analysed.

In **chapters 6** and **7** I have gone through the knowledge of an innovative technology, commonly known as 3D printing. First of all, in chapter 6, I have introduced the principles of this technology, focusing in particular on the most important advances of the application of 3D printing for cereal based products. I reviewed some of the aspect which are of utmost importance by affecting the printability of a food formula. In chapter 7 I adopted 3D printing technology to obtain innovative and personalised snack, nutritionally designed to meet the micronutrient requirements of women.

The last chapter (**chapter 8**) gave a final conclusion and a general discussion of the fundamental findings of this thesis.

References

Afify A.E.M.M.R., El-Beltagi H.S., Abd El-Salam S.M., & Omran A.A. (2015). Effect of Soaking, Cooking, Germination and Fermentation Processing on Physical Properties and Sensory Evaluation of Sorghum Biscuits. *Notulae Scientia Biologica,* 7(1) doi:10.15835/nsb719428.

Alaunyte I., Stojceska V., Plunkett A., Ainsworth P., & Derbyshire E. (2012). Improving the quality of nutrient-rich Teff (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough breadmaking. *Journal of Cereal Science, 55*(1), 22-30. doi: http://dx.doi.org/10.1016/j.jcs.2011.09.005

Araya A., Keesstra S.D., & Stroosnijder L. (2010). Simulating yield response to water of Teff (Eragrostis tef) with FAO's AquaCrop model. *Field Crops Research, 116*, 196–204.

Asharaf M., Rashed M.G., & Gibson, I. (2018). Challenges and prospects of 3D Printing in structural engineering. *13th International Conference on Steel, Space and Composite Structures*, Perth, Australia.

Ashwell M.M., Howarth E., Chesters D., Allan P., Hoyland A., & Walton J. (2014). A web-based weight loss programme including breakfast cereals results in greater loss of body mass than a standardised web-based programme in a randomised controlled trial. *Obesity Facts, 7,* 361-375.

Baye K. (2014) Teff: nutrient composition and health benefits. *Working Paper Internationl Food Policy Research Institute., 67,* 1-17.

Berrios J.J., Ascheri J.L.R., & Losso J.N. (2013). Extrusion Processing of Dry Beans and Pulses. In Siddiq M. & Uebersax M.A., *Dry Beans and Pulses Production, Processing and Nutrition,* UK: Wiley-Blackwell, 185–203.

Bose S., Vahabzadeh, S., & Bandyopadhyay, A. (2013). Bone tissue engineering using 3D Printing. *Materials Today*, 16, 496-504.

Campo E., del Arco L., Urtasun L., Oria R., & Ferrer-Mairal A. (2016). Impact of sourdough on sensory properties and consumers' preference of gluten-free breads enriched with teff flour. *Journal of Cereal Science, 67*, 75–82.

Cavaliere A., Ricci E.C., & Banterle A. (2015). Nutrition and health claims: Who is interested? An empirical analysis of consumer preferences in Italy. *Food Quality and Preference, 41,* 44–51. Doi: http://dx.doi.org/10.1016/j.foodqual.2014.11.002.

Cho S., Dietrich M., Brown C.J., Clark C.A., Block G. (2003). The effect of breakfast type on total daily energy intake and body mass index: results from the Third National Health and Nutrition Examination Survey NHANES III. *Journal of the American College of Nutrition, 22,* 296–302.

Clark C.A., Gardiner J., McBurney M.I., Anderson M., Weatherspoon L.J., Henry D.N. & Hord N.G. (2006). Effect of breakfast meal composition on second meal metabolic responses in adult with type 2 diabetes mellitus. *European Journal of Clinical Nutrition, 60,* 1122-1129.

De la Hunty A., Gibson S., & Ashwell M. (2013). Does regular cereal consumption help childern and adolescents stay smiller? A systematic review and meta-analysis. *Obesity Facts, 6,* 70-85.

De Obaldia E.E., Jeong C., Grunenfelder L.K., Kisailus, D., & Zavattieri, P. (2015). Analysis of the mechanical response of biomimetic materials with highly oriented microstructures through 3D printing, mechanical testing and modeling. *Journal of mechanical behavior of biomedical materials*, 48, 70-85.

Derossi A., Caporizzi R., Azzollini D., Severini C. (2018) Application of 3D printing for customized food. A case on the development of a fruit-based snack for children. *Journal of Food Engineering, 220*, 65-75.

Galindo A., Collado-González J., Griñán I., Corell M., Centeno A., Martín-Palomo M.J., . . . López-Pérez D. (2017). Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agricultural Water Management*. doi: https://doi.org/10.1016/j.agwat.2017.08.015

Gebremariam M.M., Zarnkow M., & Becker T. (2014). Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. *Journal of Food Science and Technology, 51*(11), 2881-2895. doi: 10.1007/s13197-012-0745-5

Gibson S. (2003). Micronutrient intakes, micronutrient status and lipid profiles among young people consuming different amounts of breakfast cereals: further analysis of data from the National Diet and Nutrition Survey of Young People aged 4 to 18 years. *Public Health Nutrition, 6,* 815–820.

Giuberti G., Gallo A., Fiorentini L., Fortunati P., & Masoero F. (2016). In vitro starch digestibility and quality attributes of gluten free 'tagliatelle' prepared with teff flour and increasing levels of a new developed bean cultivar. *Starch/Stärke, 68*, 374–378.

Goyanes A., Buanz A.B.M., Basit A.W., & Gaisford S. (2014). Fused-filament 3D printing (3DP) for fabrication of tablets. *International Journal of Phermaceutics*, 476, 88-92.

Hager A.S., Czerny M..B.J., Zannini E., & Arendt E.K. (2013). Starch properties, in vitro digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour. *Journal of Cereal Science, 58*, 156–163.

Hager A.S., Lauck F., Zannini E., & Arendt E.K. (2012). Development of gluten-free fresh egg pasta based on oat and teff flour. *European Food Research and Technology, 235*, 861–871.

Haileselassie H., Araya A., Habtu S., Meles K.G., Gebru G., Kisekka I., . . .& Foster A.J. (2016). Exploring optimal farm resources management strategy for Quncho-teff (Eragrostis tef (Zucc.) Trotter) using AquaCrop model. *Agricultural Water Management, 178*, 148-158. doi: https://doi.org/10.1016/j.agwat.2016.09.002

Hao L., Mellor S., Seaman O., Henderson J., Sewell N., & Sloan, M., 2010. Material characterisation and process development for chocolate additive layer manufacturing. *Virtual and Physical Prototyping,* 5(2), 57-64.

Hamilton C.A., Alici G., & Panhuis M. (2018). 3D printing Vegemite and Marmite: Redefining "breadboards". *Journal of Food Engineering, 220*(C), 83-88. doi: https://doi.org/10.1016/j.jfoodeng.2017.01.008

Harris J.L., Schwartz M.B., Sarda V., Dembek C., Munsell C., Shin C., Ustjanauskas A. & Weinberg M. (2012). Cereals Facts, Rudd Centre for Food Policy and Obesity

Holland S., Foster T., MacNaughtan W., & Tuck C. (2018). Design and characterisation of food grade powders and inks for microstructure control using 3D printing. *Journal of Food Engineering, 220*(C), 12-19. doi: https://doi.org/10.1016/j.jfoodeng.2017.06.008

Hwa L.C., Rajoo S., Noor A.M., Ahmad N., & Uday M.B. (2017). *Current opinion in solid state and materials science, 21*(6), 323-347.

Ibanoglu S, Ainsworth P., A.E. Ozer, & Plunkett A. (2006) Physical and sensory evaluation of a nutritionally balanced gluten-free extruded snack *Journal of Food Engineering 75,* 469–472.

Inglett G.E., Chen D., & Liu S.X. (2016). Physical Properties of Gluten Free Sugar Cookies Containing Teff and Functional Oat Products. *Journal of Food Research,* 5(3). doi: 10.5539/jfr.v5n3p72.

Lacey T., & Carol L. (2005). Eragrostis Teff as a Specialised Niche Crop. Government of Western Australia, Department of Agriculture Farmnote. 42

Lee C.M. & Lee S.Y. (2007). Consumer insights on healthy breakfast cereal- A focus group research. *Journal of Sensory Studies, 22*, 417–432.

Le Tohic C., O'Sullivan J.J., Drapala K P., Chartrin V., Chan T., Morrison A.P., . . .& Kelly A.L. (2018). Effect of 3D printing on the structure and textural properties of processed cheese. *Journal of Food Engineering, 220*(C), 56-64. doi: https://doi.org/10.1016/j.jfoodeng.2017.02.003

Lipson H, Lipton J. (2013). Adventures in Printing Food. IEEE Spectrum

Lipton J.I., Cutler M., Nigl F., Cohen D., & Lipson H. (2015). Additive manufacturing for the food industry. *Trends in Food Science and Technology, 43*(1), 114-123. doi: 10.1016/j.tifs.2015.02.004

Liu Z., Zhang M., Bhandari B., & Yang C. (2018). Impact of rheological properties of mashed potatoes on 3D printing. *Journal of Food Engineering, 220*(C), 76-82. doi: https://doi.org/10.1016/j.jfoodeng.2017.04.017

Mäkilä L., Laaksonen O., Ramos Diaz J.M., Vahvaselkä M., Myllymäki O., Lehtomäki I., . . . & Kallio H. (2014). Exploiting blackcurrant juice press residue in extruded snacks. *LWT - Food Science and Technology, 57*(2), 618-627. doi: https://doi.org/10.1016/j.lwt.2014.02.005

Marti A., Marengo M., Bonomi F., Casiraghi M.C., Franzetti L., Pagani M.A., & Iametti S. (2017). Molecular features of fermented teff flour relate to its suitability for the production of enriched gluten-free bread. *LWT - Food Science and Technology, 78*, 296-302. doi: https://doi.org/10.1016/j.lwt.2016.12.042

Norberg S., Roseberg R.J., Charlton B.A., & Shock C .(2009). Teff, a New Warm-Season Annual Grass for Oregon. Oregon State University Extension Service, EM 8970-E, Corvallis, OR.

Oliveira L.C., Rosell C.M., & Steel C.J. (2015). Effect of the addition of whole-grain wheat flour and of extrusion process parameters on dietary fibre content, starch transformation and mechanical properties of a ready-to-eat breakfast cereal.

International Journal of Food Science & Technology, 50(6), 1504-1514. doi: 10.1111/ijfs.12778
Pati F., Ha D.H., Jang J., Han H.H., Rhie J.W., & Cho D.W. (2015). Biomimetic 3D tissue printing for soft tissue regeneration. *Biomaterials*, 62, 164-175.
Peressini D., Foschia M., Tubaro F., & Sensidoni A. (2015) Impact of soluble dietary fibre on the characteristics of extruded Snacks. *Food Hydrocolloids, 43*, 73-81.
Pontieri P., Troisi J., Di Fiore R., Di Maro A., Bean S.R., Tuinstra M.R., Roemer E., Boffa A., Del Giudice A., Pizzolante G., Alifano P., & Del Giudice L. (2014). Mineral contents in grains of seven food-grade sorghum hybrids grown in a Mediterranean environment. *Australian Journal of Crop Science, 8*(11), 1550-1559
Potter R., Stojceska V., & Plunkett A. (2013). The use of fruit powders in extruded snacks suitable for Children's diets. *LWT - Food Science and Technology, 51*(2), 537-544. doi: http://dx.doi.org/10.1016/j.lwt.2012.11.015
Pradeep P.M., & Sreerama Y.N. (2017). Soluble and bound phenolics of two different millet genera and their milled fractions: Comparative evaluation of antioxidant properties and inhibitory effects on starch hydrolysing enzyme activities. *Journal of Functional Foods, 35*, 682-693. doi: https://doi.org/10.1016/j.jff.2017.06.033
Proietti I., Frazzoli C., & Mantovani A. (2015). Exploiting Nutritional Value of Staple Foods in the World's Semi-Arid Areas: Risks, Benefits, Challenges and Opportunities of Sorghum. *Healthcare, 3*(2). doi: 10.3390/healthcare3020172
Queiroz V.A.V., da Silva C.S., de Menezes C.B., Schaffert R.E., Guimarães F.F.M., Guimarães L.J.M., . . .& Tardin F.D. (2015). Nutritional composition of sorghum [sorghum bicolor (L.) Moench] genotypes cultivated without and with water stress. *Journal of Cereal Science, 65*, 103-111. doi: https://doi.org/10.1016/j.jcs.2015.06.018
Robin F., Dubois C., Pineau N., Labat E., Théoduloz C., & Curti D. (2012). Process, structure and texture of extruded whole wheat. *Journal of Cereal Science, 56*(2), 358-366. doi: https://doi.org/10.1016/j.jcs.2012.02.014
Sckokai P., & Varacca **A. (**2012). Product Differentiation and Brand Competition in the Italian Breakfast Cereal Market: a Distance Metric Approach. *Bio-based and Applied Economics, 1*(3), 297-312.
Severini C., Derossi A., & Azzollini D. (2016). Variables affecting the printability of foods: Preliminary tests on cereal-based products. *Innovative Food Science & Emerging Technologies, 38*, 281-291. doi: https://doi.org/10.1016/j.ifset.2016.10.001
Severini C., Azollini D., Albenzio M., Derossi A. (2018 a). On printability, quality and nutritional properties of 3D printed cereal based snacks enriched with edible insects. *Food Research International, 106*(4), 666-676
Severini C., Derossi A., Ricci I., Caporizzi R., & Fiore A. (2018 b). Printing a blend of fruit and vegetables. New advances on critical variables and shelf life of 3D edible objects. *Journal of Food Engineering, 220*, 89-100. doi: https://doi.org/10.1016/j.jfoodeng.2017.08.025
Shahidi F., & Chandrasekara A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods, 5*(2), 570-581. doi: https://doi.org/10.1016/j.jff.2013.02.004
Solomon, W.K. (2014). Hydration kinetics of direct expanded tef flour breakfast cereals in water and milk. *Food Science & Nutrition, 2*(1), 39-45. doi: 10.1002/fsn3.80
Stojceska V., Ainsworth P., Plunkett A., & İbanoğlu Ş. (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made

from food by-products. *Food Chemistry, 114*(1), 226-232. doi: http://dx.doi.org/10.1016/j.foodchem.2008.09.043

Stojceska V., Ainsworth P., Plunkett A., & İbanoğlu Ş. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry, 121*(1), 156-164. doi: http://dx.doi.org/10.1016/j.foodchem.2009.12.024

Sun J., Zhou W., Yan L., Huang D., & Lin L. (2018). Extrusion-based food printing for digitalized food design and nutrition control. *Journal of Food Engineering, 220*(C), 1-11. doi: https://doi.org/10.1016/j.jfoodeng.2017.02.028

Taha S.E., Shahira M.E., & Amani A.S. (2012). Chemical and biological study of the seeds of Eragrostis tef (Zucc.) Trotter, *Natural Product Research*: *Formerly Natural Product Letters, 26*, 619-629. http://dx.doi.org/10.1080/14786419.2010.538924.

Taylor J.R.N., Belton P.S., Beta T., & Duodu K.G. (2014). Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science, 59*(3), 257-275. doi: https://doi.org/10.1016/j.jcs.2013.10.009

Teklehaimanot W.H., Duodu K.G., & Emmambux M.N. (2013). Maize and teff starches modified with stearic acid as potential fat replacer in low calorie mayonnaise-type emulsions. *Starch/Stärke, 65*, 773–781.

Teba C., da Silva E.M.M., Chávez D.W.H., de Carvalho C.W.P., Ascheri J.L.R. (2017) Effects of whey protein concentrate, feed moisture and temperature on the physicochemical characteristics of a rice-based extruded flour. *Food Chemistry, 228*, 287–296

Valcarcel M., Ghatak R., Bhaduri S., & Navder K.P. (2012). Physical, Textural and Sensory Characteristics of Gluten-Free Muffins Prepared with Teff Flour (eragrostis Tef (zucc) Trotter). *Journal of the Academy of Nutrition and Dietetics, 112*(9), A58. doi: http://dx.doi.org/10.1016/j.jand.2012.06.210

Wang L., Zhang M., Bhandari B., & Yang C. (2018). Investigation on fish surimi gel as promising food material for 3D Printing. *Journal of Food Engineering, 220*(C), 101-108.

Williams P.G. (2014). The Benefits of Breakfast Cereal Consumption: A Systematic Review of the Evidence Base. *Advances in Nutrition, 5*, 636S–673S, doi:10.3945/an.114.006247.

Wolter A., Hager A.S., Zannini E., & Arendt E. K. (2014). Influence of sourdough on in vitro starch digestibility and predicted glycemic indices of gluten-free breads. *Food & Function, 5*, 564–572.

Wu G., Bennett S.J., Bornman J.F., Clarke M.W., Fang Z., & Johnson S.K. (2017). Phenolic profile and content of sorghum grains under different irrigation managements. *Food Research International, 97*, 347-355. doi: https://doi.org/10.1016/j.foodres.2017.04.030

Yang F., Zhang M., Bhandari B., & Liu Y. (2018) Investigation on lemon juice gel as food material for 3D printing and optimization of printing parameters. *LWT - Food Science and Technology, 87*, 67-76.

Yihun Y.M., Haile A.M., Schultz B., & Erkossa T. (2013). Crop water productivity of irrigated teff in a water stressed region. *Water resources management, 27*, 3115-3125. Doi: 10.1007/s11269-013-0336-x

Zhu F. (2018). Chemical composition and food uses of teff (Eragrostis tef). *Food Chemistry, 239*, 402-415. doi: https://doi.org/10.1016/j.foodchem.2017.06.101

CHAPTER 2. Particle size distribution of durum wheat flour affects antioxidant capacity as measured by the QUENCHER$_{ABTS}$ assay

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Abstract

The $QUENCHER_{ABTS}$ (QUick, Easy, New, CHEap and Reproducible) assay is based on the direct reaction of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation with fine solid food particles without extraction of antioxidants. This allows not only liquid-liquid interactions occurring in the solvent between ABTS•+ molecules and soluble antioxidants, but also solid-liquid interactions between ABTS•+ and antioxidants bound to insoluble matter by surface reactions. This kind of interactions may change depending on the size of food particles; so, here analysis was performed of Antioxidant Capacity (AC) of whole flour (WF) deriving from a mix of grains of ten durum wheat (Triticum durum Desf.) varieties, but characterized by three different particle sizes: ≤0.2 mm (control, WF0.2), ≤0.5 mm and ≤1 mm (WF0.5 and WF1, respectively).

$QUENCHER_{ABTS}$ measured similar AC values in WF0.2 and WF0.5, 42.0±2.7 and 38.3±0.9 μmol eq. Trolox/g dry weight (d.w.) respectively, provided that for WP0.5 a properly adopted calculation procedure was used based on the slope value of the regression line of ABTS•+ response vs flour amount. On the contrary, WF1 showed about half AC (20.3±0.2μmol eq. Trolox/g d.w.); so, very large particle size may deeply affect AC determination by the $QUENCHER_{ABTS}$ assay. This may be explained by the strong decrease of total surface area/volume ratio due to the doubling of particle size, resulting in a lower interaction between ABTS•+ and bound antioxidants.

Keywords: Antioxidant capacity; $QUENCHER_{ABTS}$; durum wheat grains; particle size.

Abbreviations: ABTS, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); AC, Antioxidant Capacity; d.w., dry weight; f.w., fresh weight; QUENCHER, QUick, Easy, New, CHEap and Reproducible; WF, Whole Flour.

2.1 Introduction

To date, a large number of different assays for *in vitro* Antioxidant Capacity (AC) measurement of foods has been developed, all of them showing some advantages and disadvantages mainly depending on the chemical mechanism/s involved and on the oxidant species/probe(s) and techniques used to monitor the reaction (Magalhaes et al., 2008; Carocho et al., 2013). Moreover, some assays provide results of questionable physiological relevance and often not related to individual dietary antioxidants or to different phytochemicals synergically acting; these aspects are discussed in Pastore et al. (2009) and Laus et al. (2012 and 2013). From a methodological point of view, the most commonly used AC assays require the preliminary extraction of antioxidants from food matrices prior to AC measurement. Different extraction procedures are available, all of them showing several limitations: *i*) the extraction yield is largely influenced by food composition and extraction solvents; *ii*) many protocols aimed at releasing compounds bound to insoluble matter involve successive time-consuming, poorly efficient and partially recovering hydrolysis steps, also able to alter chemical structure of some antioxidant compounds; *iii*) possible synergistic interactions among antioxidants are lost when different antioxidants are separately extracted and measured; *iv*) results obtained with different extraction protocols may be different and inter-laboratory data comparison is rather difficult (Serpen et al., 2007 and 2008; Gökmen et al., 2009).

To overcome these problems, a new simple and direct procedure for AC assessment of food matrices avoiding any extraction and hydrolysis step has been proposed (Serpen et al., 2008). It involves a direct reaction of food solid particles with the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation reagent, followed by centrifugation to obtain an optically clear supernatant for absorbance measurement at 734 nm. The new approach, abbreviated as "QUENCHER" (QUick, Easy, New, CHEap and Reproducible), takes advantage from liquid-liquid interactions occurring in the solvent (usually a mixture of ethanol and water) between soluble antioxidants and $ABTS^{\bullet+}$ molecules, but, interestingly, it also may assess AC of antioxidants bound to insoluble matter by surface solid-liquid interactions (Serpen et al., 2007 and 2008; Gökmen et al., 2009). Interestingly, this direct procedure, avoiding any pretreatment of food samples, may give AC values generally higher than that obtained by different extraction and hydrolysis procedures used for comparison (Serpen et al., 2008); moreover, these values are potentially related to a true antioxidant action in food or in human gastrointestinal trait (Serpen et al., 2008; Gökmen et al., 2009), thus showing possible physiological relevance of results. The QUENCHER procedure is very versatile; in fact, it has been successfully applied to measure AC of a lot of different foods, including cereal whole grains and some milling fractions (Serpen et al., 2008; Žilić et al., 2012), fruits and vegetable (Serpen et al., 2012a), thermally processed foods (Serpen et al., 2009), bakery products and multi-grain containing breads (Ciesarova et al., 2009; Delgado-Andrade et al., 2010; Serpen et al., 2012c), roasted pulses, nuts and seeds (Acar et al., 2009), corn-based breakfast cereals (Rufian-Henares et al., 2009), raw and cooked meat samples (Serpen et al., 2012b), soybean seed coats and dehulled beans (Žilić et al., 2013), soy proteins (Amigo-Benavent et al., 2010), and even to nanoparticles (Saikia et al., 2010). QUENCHER procedure has been successfully performed also replacing $ABTS^{\bullet+}$ as radical probe with DPPH (Gökmen et al., 2009; Serpen et al., 2012a; b; c), as well as using the colour generation of FRAP (Serpen et al., 2012a and b) and CUPRAC reagents (Tufan et al., 2013) or fluorescence as in ORAC protocol (Amigo-Benavent et al., 2010; Kraujalis et al., 2013).

Obviously, in this approach, total surface area and solid food particle sizes may play a crucial role in determining the reaction rate and, in turn, the measured AC (Gökmen et al., 2009). To date, the QUENCHER procedure has been generally applied to finely ground food solid particles having generally a diameter not exceeding 0.2-0.3 mm. Nevertheless, some food matrices of interest may be produced and used as larger size particles. At this purpose, industrial milling process of wheat grains generates by-products showing different particle size distribution mainly dependent on milling process and grain hardness (Devaux et al., 1998). Interestingly, large particles may display special properties. For example, as for wheat bran, the addition of bran having larger particle size (≥0.5 mm) has been reported to positively influence technological performance, as well as quality and sensory characteristics of some integrated fiber-rich foods (Noort et al., 2010; Chen et al., 2011). Moreover, large particle-bran has been also shown to induce a greater acetate production in an *in vitro* fermentation system (Stewart et al., 2009) and to influence the extent of starch retrogradation and in turn starch digestibility in bran-enriched bread during storage (Cai et al., 2014), so suggesting that large particles may exert physiological effects.

In the light of these observations, AC determination of large particles may have physiological interest; so, the goal of this study was to test the applicability of the

$QUENCHER_{ABTS}$ protocol to solid particles having large size. This was performed by analyzing whole flour (WF) deriving from a mix of grains of ten durum wheat (*Triticum durum* Desf.) varieties, obtained by using either 0.5 mm ($WF_{0.5}$) or 1 mm (WF_1) sieves. A $WF_{0.2}$ having particle size ≤0.2 mm was used as a control.

2.2 Material and Methods

2.2.1 Chemicals

All reagents at the highest commercially available purity were purchased from Sigma-Aldrich Corp. (St. Louis, Mo., U.S.A.).

2.2.2 Plant material

Grain samples from ten durum wheat varieties (Quadrato, Torrebianca, Pietrafitta, Vendetta, Alemanno, Principe, Cannavaro, Gattuso, Simeto and Duilio) were stored under vacuum at 4°C for no longer than 2 months; before use, a balanced mix of whole grains were daily milled by means of a Cyclotec 1093 Sample Mill (using 1 mm or 0.5 mm sieves). Ground samples were consecutively passed through 1 mm, 0.5 mm and 0.2 mm certified test sieves (Giuliani Tecnologie, Turin, Italy) to obtain WF_1, $WF_{0.5}$ and $WF_{0.2}$, respectively.

2.2.3 Determination of AC by the direct $QUENCHER_{ABTS}$ procedure

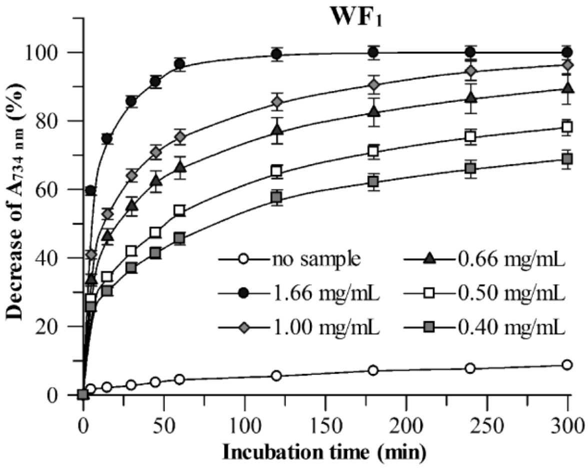
The protocol described in Serpen et al. (2008) was applied to $WF_{0.2}$ with minor modification (60 min reaction time and ratio between flour amount and volume of $ABTS^{•+}$ solution ranging from 0.10 to 0.35 mg fresh weight, f.w./mL). A properly adapted protocol was applied to WF containing large particles ($WF_{0.5}$ e WF_1). The $ABTS^{•+}$ radical cation was generated by chemical oxidation with potassium persulfate as described by Re et al. (1999) and then diluted in a mixture of ethanol:water (50:50, v/v) to obtain an A_{734} value equal to 0.70 ± 0.02. In general, measurements were carried out by adding the $ABTS^{•+}$ diluted solution with flour sample and vigorously stirring the suspension to facilitate a surface reaction between the solid particles and the $ABTS^{•+}$ reagent. After centrifugation at 9200x*g* for 2 min, optically clear supernatant was separated and A_{734} was measured. Measurements were carried out in triplicate. The (%) decrease of A_{734} measured after sample incubation with respect to A_{734} of $ABTS^{•+}$ solution was calculated. A linear dependence of the (%) decrease of A_{734} on sample amount was verified by linear regression analysis of data, and AC was obtained by comparing the slope derived by linear regression analysis with that of the Trolox-derived calibration curve. Different reaction times (ranging from 5 to 300 min for WF_1 and from 5 to 270 min for $WF_{0.5}$) and whole flour amount (ranging from 0.40 to 1.66 mg f.w./mL of $ABTS^{•+}$ solution for WF_1 and from 0.10 to 1.66 mg f.w./mL for $WF_{0.5}$) were analyzed.

2.3 Results

The goal of this study was to test the applicability of the $QUENCHER_{ABTS}$ procedure to solid particles having size higher than 0.2 mm. To this purpose, possible methodological adaptation of classical $QUENCHER_{ABTS}$ assay was evaluated, with particular attention to both reaction time and flour amount. So, WF_1 and $WF_{0.5}$ were analyzed by carrying out the reactions for increasing incubation times and using increasing amounts of flour sample (see also Methods). Results relative to WF_1 are shown in detail (Figs. 2.1 and 2.2). In Figure 2.1 the profile of (%) decrease of $A_{734\ nm}$ *vs* the incubation time is reported for each tested amount of WF_1, as well as that relative to the $ABTS^{•+}$ diluted solution in the absence of sample.

Figure 2.1 Dependence of the absorbance decrease (%), measured at 734 nm by using the $QUENCHER_{ABTS}$ assay, on the reaction time of the $ABTS^{•+}$ radical cation with durum wheat whole flour (≤1 mm particle size, WF_1). Measurements were carried out as described in Methods, by reacting the $ABTS^{•+}$ diluted solution with different amounts (f.w.) of WF_1 mix for different times. Data are reported as mean value ± SD (n=3 different experiments).

Fig. 1

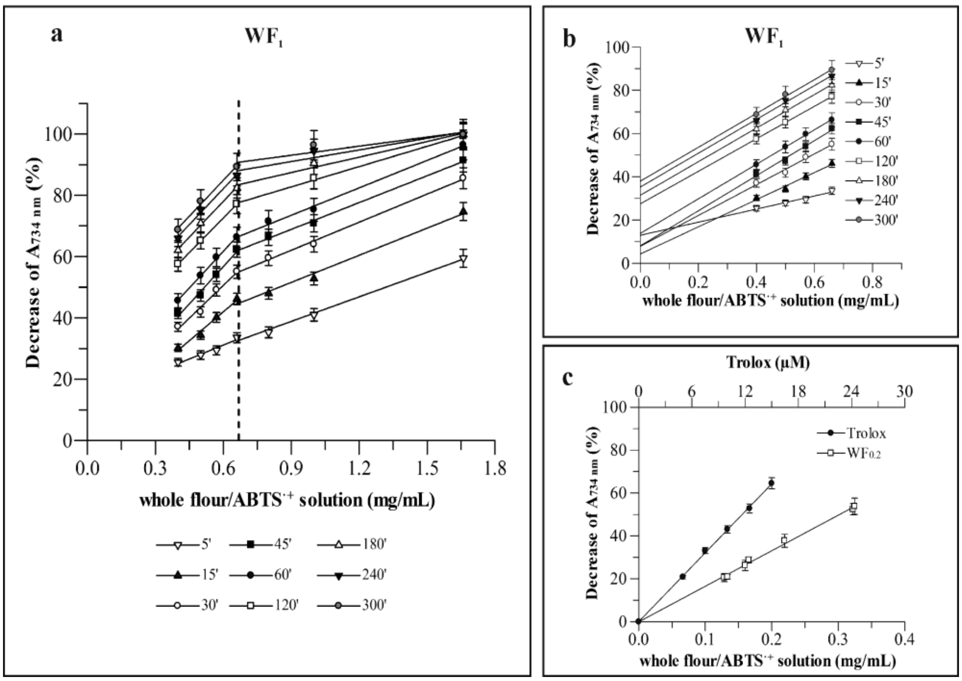


An undesired complete bleaching of the $ABTS^{•+}$ reagent was observed in the presence of 1.66 mg flour/mL solution, a condition that prevents AC evaluation under our experimental conditions. Using lower flour amounts, $ABTS^{•+}$ quenching curves were obtained showing a similar profile but, as expected, tending to a different plateau; anyhow, all conditions below 1.66 mg flour/mL solution remained below saturation of the $ABTS^{•+}$ bleaching, being able to allow AC quantification.

To define the most appropriate incubation time and ratio between flour amount and volume of $ABTS^{•+}$ reagent, data of Figure 2.1 for each incubation time were plotted as (%) decrease of $A_{734\ nm}$ *vs* the flour amount/$ABTS^{•+}$ volume (Figure 2.2a).

Figure 2.2 Dependence of the absorbance decrease (%), measured at 734 nm by using the QUENCHER$_{ABTS}$ assay, on the amount of durum wheat whole flour (≤1 mm, WF1, and ≤0.2 mm, $WF_{0.2}$). In (**a**) the curves obtained by two separate linear regression analyses of data in the 0.40-0.66 and 0.66-1.66 mg WF_1 (f.w.)/mL of $ABTS^{•+}$ solution ranges are shown. In (**b**) the extrapolation to y-axis of the curves obtained by linear regression analysis of data in the 0.40-0.66 mg/mL range is shown. In (**c**) the straight line obtained using the QUENCHER$_{ABTS}$ assay on mix of $WF_{0.2}$ and calibration curve obtained with Trolox, are reported. Data are reported as mean value ± SD (n=3 different experiments).

Fig. 2



Interestingly, two distinct flour amount ranges were found in which a different relationship was observed: 0.40-0.66 and 0.66-1.66 mg whole flour/mL $ABTS^{•+}$. This was confirmed by two separate linear regression analyses of data in these two ranges, that generated curves with much higher slopes in the 0.4-0.66 mg whole flour/mL range, and these differences in slope between the two ranges became more and more pronounced with increasing incubation time. The first range appears to be preferred to calculate AC values, since all straight lines obtained by linear regression analysis in this range, except that obtained for 5 min reaction, remained parallel whatever the reaction time (Figure 2.2b); moreover, all these straight lines showed intercepts to the y-axis nearer to the origin of the axes, which is the theoretical intercept, with respect to the regression curves obtained in the 0.66-1.66 mg/mL range. Consistently, the straight line obtained using the $QUENCHER_{ABTS}$ protocol applied to $WF_{0.2}$, as well as the calibration curve obtained with Trolox, always intercepted y-axis close to zero (Figure 2.2c).

In the light of this, y-axis intercepts obtained using the $WF_{0.5}$ and WF_1 have to be adequately subtracted in AC calculation. This may be easily made by calculating the equation of the linear regression curve and using only the slope to calculate AC value by means of a comparison with that of Trolox calibration curve. The results are reported in Table 2.1, in which equations of the linear regression curves are reported, obtained for each incubation time with either WF_1 (Fig. 2b) and $WF_{0.5}$ (data not shown), as well as the corresponding AC values referred to flour amount (μmol eq. Trolox/g of dry weight, d.w.).

AC values of WF_1 were found to increase when reaction time increased from 5 to 45 min, while they remained almost similar from 45 to 300 min incubation. The highest values were obtained at 45 and 60 min, but the latter was affected by a lower (about 1%) experimental error. In light of these results, 60 min was chosen as the most suitable reaction time. As for $WF_{0.5}$, the linear regression analysis of data in the linearity range generated curves with higher slopes, up to about a doubling. Interestingly, also these straight lines did not pass through axis origin, although lower y-intercepts were observed up to about a halving. AC values obtained by the "slope" calculation mode resulted up to about 2-fold higher than that measured for WF_1. AC value obtained at 60 min was found to be the highest and statistically equal to that measured at higher incubation times. Finally, $WF_{0.2}$ displayed an AC value equal to 42.0±2.7 (n=3) μmol eq. Trolox/g d.w. calculated as reported in Figure 2.2c.

Table 2.1. Antioxidant Capacity (AC) evaluated by the QUENCHER$_{ABTS}$ assay of durum wheat whole flour having ≤1 mm (WF_1) and ≤0.5 mm ($WF_{0.5}$) particle sizes.

WF_1		
Time of incubation (min)	**Equation of straight line**	**AC* QUENCHER$_{ABTS}$**
5	y = 29.60x + 13.25	7.7 ± 0.3
15	y = 61.31x + 4.96	16.0± 0.5
30	y = 69.51x + 8.63	18.1 ± 0.5
45	y = 79.24x + 8.90	20.7 ± 0.5
60	y = 77.86x + 14.72	20.3 ± 0.2
120	y = 73.20x + 28.36	19.1 ± 0.1
180	y = 75.54x + 32.36	19.7 ± 0.4
240	y = 76.04x + 36.21	19.8 ± 0.5
300	y = 76.09x + 38.94	19.8 ± 0.5
$WF_{0.5}$		
Time of incubation (min)	**Equation of straight line**	**AC* QUENCHER$_{ABTS}$**
5	y = 72.86x + 4.04	20.2 ± 0.4
15	y = 90.82x + 6.23	25.2 ± 0.6
30	y = 116.7x + 6.26	32.4 ± 0.8
45	y = 123.30x + 8.10	34.3 ± 0.7
60	y = 137.95x + 9.07	38.3 ± 0.9
120	y = 138.57x + 13.45	38.5 ± 1.2
180	y = 140.30x + 17.41	39.0 ± 1.5
270	y = 138.28x + 19.16	38.4 ± 1.3

Equations of straight lines obtained by the linear regressions of data relative to WF_1 (Fig. 2b) are reported, as well that obtained using $WF_{0.5}$. particles in the 0.10-0.35 mg/mL range. AC obtained by QUENCHER$_{ABTS}$ was calculated comparing the slope of each straight line with that of Trolox calibration curve (Fig. 2.2c).

*Data are expressed as μmol eq. Trolox/g d.w. and reported as mean value ± SD (n=3 different experiments).

2.4 Discussion

The QUENCHER$_{ABTS}$ method allows for AC evaluation of compounds without preliminary extraction when they are still bound to the insoluble food matrix. Recently, we have shown that in whole flour of durum wheat the QUENCHER$_{ABTS}$ assay highlights AC mainly due to bound phenols (unpublished data). This is a considerable usefulness that also may prevent some misjudgements; for example, acid hydrolysis of bound phenolic compounds may produce 5-hydroxymethyl-2furfural and derivatives, able to display AC, that may induce an incorrect AC determination when extracts are analyzed (Chen et al., 2014).

As for AC measurements of cereal grains by using this direct procedure, it has been reported that an accurate grinding is required to obtain particles having a diameter ranging between 0.1 and 0.3 mm (Gökmen et al., 2009). Nevertheless, since the use of larger particles of some cereal milling products has been reported to exert positive effect on technological performance and quality of some derived foods (Noort et al., 2010; Chen et al., 2011), as well as to induce some physiological effects (Stewart et al., 2009; Cai et al., 2014), the study of AC of larger particles may be of interest. Here, we show that adopting little changes with respect to the original $QUENCHER_{ABTS}$ method, it is possible to analyze large particles up to 0.5 mm, without mistakes in measured AC values. This result extends the findings of Serpen et al. (2008), who found no relevant changes (within 20%) in AC value in the 0.105-0.177 mm particle size range. In particular, the observed feature of large particles to generate straight lines that do not pass through origin appears to be a characteristic of the QUENCHER procedure. Under these conditions, the calculation procedure based on comparison between slopes is strongly advisable to avoid AC overestimation, while classical AC measurement through interpolation would lead to incorrect values. So, our $QUENCHER_{ABTS}$ approach extends the potentiality of the QUENCHER procedure and improves its range of applicability. On the contrary, as expected in the light of the strong total surface area/volume ratio decrease due to the doubling of particle size, the AC measured is much lower when particles up to 1 mm were analyzed. So, when analysis of so large particles is carried out, this should be properly highlighted; in fact, when comparing literature AC data obtained by means of the QUENCHER procedure, it is very important to know the adopted experimental conditions with particular attention to particle size.

2.5 Conclusions

In conclusion, large size of particles of whole flour of durum wheat may strongly affect AC measured by $QUENCHER_{ABTS}$. AC of large particles up to 0.5 mm may be analyzed without relevant alteration of results by adopting a mode to calculate AC based on the slope value of the regression line of $ABTS^{\bullet+}$ response *vs* flour amount. On the contrary, AC of larger particles may result strongly underestimated, suffering of unfavorable surface-liquid reaction.

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References

Acar, O.C., Gökmen, V., Pellegrini N., Fogliano, V. 2009. Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. Eur Food Res Technol **229**: 961–969.

Amigo-Benavent, M., del Castillo M.D., Fogliano, V. 2010. Are the major antioxidants derived from soy protein and fructo-oligosaccharides model systems colored aqueous soluble or insoluble compounds? Eur Food Res Technol **231**: 545–553.

Cai, L., Choi, I., Hyun, J.N., Jeong, Y.K., Baik, B.K. 2014. Influence of bran particle size on bread-baking quality of whole grain wheat flour and starch retrogradation. Cereal Chem **91**: 65–71.

Carocho, M.,Ferreira, I.C.F.R. 2013. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol **51**: 15–25.

Chen, J.S., Fei, M.J., Shi, C.L., Tian, J.C., Sun, C.L., Zhang, H., Ma, Z., Dong, H.X. 2011. Effect of particle size and addition level of wheat bran on quality of dry white Chinese noodles. J Cereal Sci **53**: 217–224.

Chen, P.X., Tang, Y., Zhang, B., Liu, R., Marcone, M.F., Xihong, L., Tsao, R. 2014. 5-hydroxymethyl-2-furfural and derivatives formed during acid hydrolysis of conjugated and bound phenolics in plant foods and the effects on phenolic content and antioxidant capacity. J Agric Food Chem **62**: 4754–4761.

Ciesarova, Z., Kukurova, K., Bednarikova, A., Morales, F.J. 2009. Effect of heat treatment and dough formulation on the formation of Maillard reaction products in fine bakery products-benefits and weak points. J Food Nutr Res **48**: 20–30.

Delgado-Andrade C., Conde-Aguilera J.A., Haro A., Pastoriza de la Cueva S., Rufian-Henares J.A. 2010. A combined procedure to evaluate the global antioxidant response of bread. J Cereal Sci **52**: 239–246.

Devaux, M.F., Le Deschault de Monredon F., Guibert D., Novales B., Abecassis J. 1998. Particle size distribution of break, sizing and middling wheat flours by laser diffraction. J Sci Food Agric **78**: 237–244.

Gökmen, V., Serpen, A., Fogliano, V. 2009. Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. Trends Food Sci Tech **20**: 278–288.

Kraujalis, P., Venskutonis, P.R., Kraujalienė, V., Pukalskas, A. 2013. Antioxidant properties and preliminary evaluation of phytochemical composition of different anatomical parts of amaranth. Plant Foods Hum Nutr **68**: 322–328.

Laus, M.N., Soccio, M., Pastore, D. 2013. Evaluation of synergistic interactions of antioxidants from plant foods by a new method using soybean lipoxygenase. J Food Nutr Res **52**: 256–260.
Laus, M.N., Tozzi, D., Soccio, M., Fratianni, A., Panfili, G., Pastore, D. 2012. Dissection of antioxidant activity of durum wheat (*Triticum durum* Desf.) grains as evaluated by the new LOX/RNO method. J Cereal Sci **56**: 214–222.
Magalhaes, L.M., Segundo, M.A., Reis, S., Lima, J.L.F.C. 2008. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal Chim Acta **613**: 1–19.
Noort, M.W.J., van Haaster, D., Hemery, Y., Schols, H.A., Hamer, R.J. 2010. The effect of particle size of wheat bran fractions on bread quality - Evidence for fibre-protein interactions. J Cereal Sci **52**: 59–64.
Pastore, D., Laus, M.N., Tozzi, D., Fogliano, V., Soccio, M., Flagella, Z. 2009. New tool to evaluate a comprehensive antioxidant activity in food extracts: bleaching of 4-nitroso-*N,N*-dimethylaniline catalyzed by soybean lipoxygenase-1. J Agric Food Chem **57**: 9682–9692.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med **26**: 1231–1237.
Rufian-Henares, J.A., Delgado-Andrade, C. 2009. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. Food Res Int **42**: 394–400.
Saikia, J.P., Paul, S., Konwar, B.K., Samdarshi, S.K. 2010. Nickel oxide nanoparticles: a novel antioxidant. Colloids Surf B **78**: 146–148.
Serpen, A., Gökmen, V. 2009. Evaluation of the Maillard reaction in potato crisps by acrylamide, antioxidant capacity and color. J Food Compos Anal **22**: 589–595.
Serpen, A., Capuano, E., Fogliano, V., Gökmen, V. 2007. A new procedure to measure the antioxidant activity of insoluble food components. J Agri Food Chem **55**: 7676–7681.
Serpen, A., Gökmen, V., Fogliano, V. 2012a. Solvent effects on total antioxidant capacity of foods measured by direct QUENCHER procedure. J Food Compos Anal **26**: 52–57.
Serpen, A., Gökmen, V., Fogliano, V. 2012b. Total antioxidant capacities of raw and cooked meats. Meat Sci **90**: 60–65.
Serpen, A., Gökmen, V., Mogol, B.A. 2012c. Effects of different grain mixtures on Maillard reaction products and total antioxidant capacities of breads. J Food Compos Anal **26**: 160–168.
Serpen, A., Gökmen, V., Pellegrini, N., Fogliano, V. 2008. Direct measurement of the total antioxidant capacity of cereal products. J Cereal Sci **48**: 816-820.
Stewart, M.L., Slavin, J.L. 2009. Particle size and fraction of wheat bran influence short-chain fatty acid production *in vitro*. Brit J Nutr **102**: 1404–1407.
Tufan, A.N., Celik, S.E., Ozyurek, M., Guclu, K., Apak, R. 2013. Direct measurement of total antioxidant capacity of cereals: QUENCHER-CUPRAC method. Talanta **108**: 136–142.
Žilić, S., Akillioglu, H.G., Serpen, A., Peric, V., Gökmen, V. 2013. Comparison of phenolic compounds, isoflavones, antioxidantcapacity and oxidative enzymes in yellow and black soybeans seed coat and dehulled bean. Eur Food Res Technol **237**: 409–418.
Žilić, S., Serpen, A., Akıllıoglu, G., Jankovic, M., Gökmen, V. 2012. Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J Cereal Sci **56**: 652–658.

CHAPTER 3. Evaluating the effect of deficit irrigation on some quality parameters of millet sorghum and teff.

Abstract

Minor cereals are attracting much interest in recent years because of their nutritional and functional properties. In addition they are more tolerant to biotic and abiotic stress, so they could be used in a Mediterranean region, which is generally characterized by scarce rainfall and hot dry summer. Millet, sorghum and teff were grown in a Mediterranean area under full and deficit irrigation with the aim to evaluate the agronomical response of these crops, as well as to investigate their antioxidant capacity.

Irrigation treatment significantly affected yield ($p<0.05$), and bound phenolic content ($p<0.001$). High differences were observed among the species investigated on all the parameters analyzed. In particular sorghum exhibited the highest yield and free phenolic content; millet showed the highest bound phenolic content but the lowest free phenolic content and total antioxidant capacity. Teff was probably the well adapted crop because of its best yield performance under deficit irrigation and the high antioxidant capacity.

Keywords: teff, millet, sorghum, deficit irrigation, total antioxidant capacity, healthy quality

3.1 Introduction

Cereals are a staple food around all over the world and contribute in a significant amount to the energy, protein and some micronutrients intake in the diet of the populations (Shahidi & Chandrasekara 2013). Even if the most important cereals in the world are, both economically and for their production, wheat, rice, maize, barely, there are some other cereal grains, known as minor cereals, which are attracting growing interest in the last years (Wu et al., 2107a), such as sorghum, millet and teff.

Sorghum is the fifth most valuable cereal crop of the world (Wu 2017b), belonging to the family *Graminaceae* or *Poaceae*, including both wild and cultivated varieties (Afyfi et al., 2015). It is grown mainly for animal feed, for the extraction of syrup and sugars, while its consumption for humans has being explored recently (Afify et al., 2015; Queiroz 2015). It is a C_4 plant, rich in minerals and vitamins, and it is a basic staple food of rural communities, especially in Africa (Pontieri 2014; Proietti et al., 2015). Millets are a heterogeneous group of small-grained cereals which are considered as the first domesticated cereals (Shahidi & Chandrasekara, 2013). They are used as food, animal feed and fodder in various parts of the world((Shahidi & Chandrasekara, 2013). As for sorghum, millets belong to the family *Poaceae*, but with different tribes and genera (Shahidi & Chandrasekara, 2013). Nutritionally they are equivalent to other cereals, except for their higher content of fat, iron and phosphorus (Shahidi & Chandrasekara, 2013). Teff [*Eragrostis tef* (Zucc.) Trotter] is a C_4 annual tropical crop in the *Poaceae* family (Zhu, 2018; Roseberg et al., 2005). It is believed to have originated in Ethiopia, where it is primarily used to make a fermented, sour dough type, flat bread called Injera (Yihun et al., 2013). It is considered the smallest cereal of the world, taking 150 grains to weight as much as one grain of wheat (Haileselassie et al., 2016; Lacey & Carol, 2005). It has an excellent nutrient profile, in particular it contains all the 8 essential amino acids for humans and its lysine content is higher than in other most common cereals, such as wheat and barley (Inglett et al., 2016; Norberg et al., 2009).

These minor cereals are achieving resounding success in the market of developing as well as developed countries as organic/healthy foods and multigrain food products (Pradeep & Sreerama, 2017). They are rich in many "health-promoting" phytochemicals, so they are an important source of dietary antioxidant (Taylor et al. 2014; Dlamini et al., 2007). This is because they are always consumed as a whole grains so they are rich of polyphenols, which are mainly concentrated in the pericarp, hull and aleurone layers (Chandrasekara et al., 2012,; Afify et al., 2012; Zhu 2018). In addition they are gluten free grains so they are well suited to meet the demands of gluten-free products (Inglett et al., 2016; Queriroz et al., 2015; Taylor et al., 2014) .

Last but not least they contribute to the food security and health in at-risk communities in Africa, South America and Asia because of their adaptability to harsh environmental conditions (Taylor et al., 2014). They widely grown in semi-arid and arid regions of the world because they are highly tolerant to drought and high temperature (Wu 2017; Queiroz 2015). They are important subsistence crops due to their sustainable yields in lands with declined soil fertility and low rainfall agro-ecosystem (Pradeep 2017; Proietti et al., 2015;). Other advantages are high resistance to pest and disease, short growing season which contribute to their high drought tolerance (Pradeep 2017, Proietti et al., 2015).

There has been only limited research on the agronomical and physiological response of these three species with water deficit in a Mediterranean region. Mediterranean climate is characterized by hot dry summer and scarce rainfall, which have to be supplemented by irrigation in order to avoid plant water deficit (Galindo et al., 2017). Indeed, water scarcity and drought are projected to increase in these regions, because of the global climate changes (Farrè & Faci, 2006). An useful strategy to contribute to water saving should be directed to the use of plant materials that are less-water demanding or that are able to withstand to deficit irrigation with a minimum impact on yield and other quality parameters (Galindo et al., 2017). It is known as water deficit usually leads to a decrease in the plant growth and crop productivity (Coyago-Cruz et al., 2018) while it can increase phenolics and other antioxidant compounds which are involved in the plant defence against biotic and abiotic stress and significantly contribute to the antioxidant capacity of a plant tissue (Ahmed et al., 2015).

The hypothesis of this study was that under water availability limited conditions sorghum, millet and teff might be a possible alternative crop in South Italy under Mediterranean environment, where durum wheat is largely cultivated. For these reasons a field experiment was performed to compare sorghum, millet and teff response to deficit irrigation in relation to both agronomical and healthy aspects. Yield parameters, lipid, protein and phenolic content and total antioxidant capacity were characterized for all the species under study.

3.2 Materials and methods

3.2.1 Field experiment and irrigation management

The experiment was carried out during the summer 2015 at Foggia (41°28′ N, 15°32′ E and 75 m a.s.l), in southern Italy, on a silt-loam soil. The soil had 1 g kg^{-1} of total nitrogen, 690 g kg^{-1} of available phosphorus, pH 7.8. The three species investigated were white sorghum (accession CRA100.100), white millet (accession CRA200.001), teff (accession CRA 300.005). The experiment was arranged in a split-plot design with treatment as the main plot and species as the sub-plot, with three replicates. Each plot was 12.5x0.35 m with a distance between plots of 1.5 m. In each plot were sowed 70 plants, divided in two rows. All the three species were sowed the 7th May 2015 and replanted 6 day after the sowing. Then the plots were covered with a PVC tarp and irrigated under the two treatments conditions with a drip irrigation. In full irrigation treatment soil water content was maintained to field capacity by applying a total water volume of 2320, 2120 and 2080 m^3/ha for sorghum, millet and teff, respectively. In the deficit irrigation treatment soil water content was maintained at 55% of field capacity.

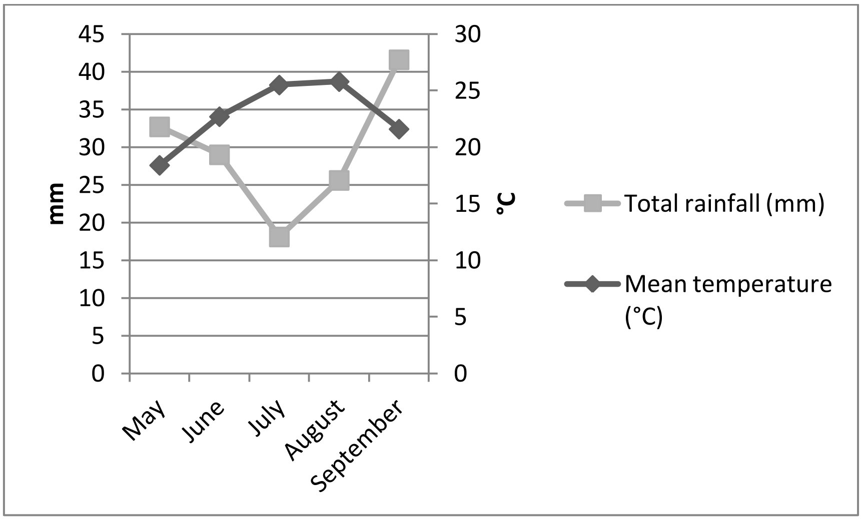
Nitrogen as Urea was applied in a split: 36 Kg N/ha before the sowing and 60 Kg N/ha 1 month after sowing. 92 Kg P/ha of ammonium phosphate, P_2O_5, was totally applied in a single moment before the sowing.

Each species was harvested in three different moments because of the differences in the maturity stages. Millet was harvested in the middle of July, Sorghum on 8th September and teff 1st October 2015. After the harvest, plant height was measured on all the plant.

Climate data including rainfall, mean temperature during the growing season were obtained from the meteorological station near the experimental field (Sperimental station of forage crops, APRE, Puglia).

The total rainfall and the mean temperature of the crop season were reported in Figure 3.1. The mean temperature varied between 18.4 in May to 25.8 in August. July and August were the months with the highest mean temperature and the lowest rainfall.

Figure 3.1. Climate data from May to September 2015 (Experimental Station of forage croprs, APRE, Puglia).



3.2.3 Chemical analysis

3.2.3.1 Extraction of bioactive compounds

Extraction of free and bound phenolics were developed with the method of Abguri et al. (2015) with some minor modifications. 0.5 g of each samples were added with methanol and each tube containing samples were closed under N_2 to avoid oxidation of bioactive compounds. Then all samples were vortexed for 20 seconds, incubated for 5 minutes at 30°C in an ultrasound bath Baudeline Sonorex RK100H (Germany), then they were overshaked for additional 5 minutes. At the end, the samples were centrifuged for 10 minutes at 4000 rpm and 4°C using a centrifuge mod. 5810R (Hamburg, Germany). The supernatant was collected as free extract and stored at -18°C until use. The solid residues were used to extract bound phenolic compounds. The residues were subjected to basic hydrolysis by adding 3 ml of distilled water, 5 ml of NaOH 5M and 5 mL of NaOH 10 M and overshaked overnight in the dark with a MultiRotator Grant-Bio PTR-60 (Cambs, England). After this step the pH was adjusted to~2 with HCl 8 M and it was added with 15 mL of ethylacetate and then centrifuged at 4000 rpm for 10 minutes 3 times in order to recover the supernatant. The latter was filtered with Na_2SO_4 and then evaporated to dryness under vacuum at 40°C. The fraction of phenolic compounds so obtained were reconstituted in methanol 50% (v/v) and stored under nitrogen atmosphere at -18°C until used for further analysis. During all stages, extract were protected from light by covering the containers with aluminium foil. Free and bound extracts were used for the determination of phenolic compounds.

3.2.3.2 Total Phenolic Content (TPC)

The total phenolic content (TPC) of each phenolic fraction was determined using the colorimetric Folin-Ciocalteau method described by Singleton and Rossi (1965) with slight modification as reported by Laus et al. (2012). The phenolic content was quantified by external calibration using gallic acid as standard. The samples for both free and bound fractions were independently analyzed in triplicates and results were expressed as μmol eq. of gallic acid per grams of grains on dry weight.

3.2.3.3 Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) was determined by the QUENCHER-ABTS assay as described by Serpen et al. (2008) and as modified by Di Benedetto et al. (2015) and Laus et al. (2015). The $ABTS^{\bullet +}$ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation was generated by chemical oxidation with potassium persulfate and then diluted in a mixture of ethanol:water (50:50, v/v) to obtain an absorbance value at 734 nm (A734) equal to 0.70±0.02. In general, measurements were carried out by adding the ABTS•+ diluted solution with whole flour sample and vigorously stirring the suspension for 60 min. After centrifugation at 9200×g for 2 min, optically clear supernatant was separated and A734 was measured. The (%) decrease of A734 measured after sample incubation with respect to A734 of ABTS•+ solution was calculated. A linear dependence of the (%) decrease of A734 on sample amount was verified by linear regression analysis of data. The analysis were carried out in triplicate on all the samples.

3.2.3.4 Lipid and protein content

Lipid content was determined according to AACC official method 30-25.01 (1999) using an automating solvent extractor Ser 148 (VELP Scientifica, Usmate, Italy) and using a petroleum ether as a solvent.

Grain protein concentration was determined by Kjeltec 2300 Analyzer Unit (FOSS Analytical, Denmark). Protein content (%) on dry weight was obtained by multiplying the total N grain % with a factor of 6.25 %. The analysis of protein and lipid content were carried out in triplicates for all the samples.

3.2.4 Statistical analysis

For all the investigated parameters, the main effect of species and irrigation treatment and their interaction were analyzed using a factorial ANOVA with Duncan's post-hoc test by a Statistica software ver. 10 (Statsoft, Tulsa, USA). The significant differences were considered at $p \leq 0.05$.

In addition a correlation matrix across all the variables was run. Pearson correlation coefficient was obtained and the significance of the correlation estimates were assessed by t-test.

3.3 Results and discussion

3.3.1 Yield parameters

Plant height was negatively affected by deficit irrigation only in sorghum which showed a decrease of about 17% in less irrigated plants respect to irrigated ones (Table 3.1). Other studies revealed the same negative trend of this parameter in response to water stress (Mansour et al., 2017; Yau et al., 2011; Seghatoleslami et al., 2008). Samarah (2001) attributed to the reduction of plant height in barley exposed to drought stress, to lower gross photosynthetic rate and to the decrease of the osmotic potential. Statistical analysis also detected significant differences among the three species investigated ($p<0.001$)

(Table 3.3) with teff showing the highest value (about 210 cm) and sorghum the lowest (Table 3.1).

Concerning yield parameters, the 1000 kernel weight significantly decreases under deficit irrigation, only in millet. (Table 3.1). On the contrary Seghatoleslami et al. (2005) observed no effect of deficit irrigation on 1000 kernel weight. Yield is an important parameter for the evaluation of the crop productivity and adaptability. As expected deficit irrigation decreased yield in millet and sorghum of about 37% in each species, while no significant differences were highlighted in teff where yield was observed to slightly increase. The yield stability observed in teff was a very interesting result on the adaptability of this crop in a Mediterranean region. This was probably due to the fact that during deficit irrigation treatment a partial irrigation was guaranteed in the initial stage of growing. Yihun et al. (2013) reported as adequate watering conditions in the first stages of the growing season could lead to an adequate yield. On the other hand the decrease in the yield of sorghum and millet was recorded in several studies (Wu et al., 2017; Queiroz et al., 2015; Seghatoleslami

et al., 2005), moreover yield values of sorghum and millet with full and deficit irrigation are in accordance with Farre & Faci (2006) and Seghatoleslami et al. (2005), respectively.
In Table 3.4 the results of the correlation analysis are reported. It is shown as plant height is negatively and significantly correlated with yield and 1000 kernel weight with values of -0.470 ($p \leq 0.05$) and -0.634 ($p \leq 0.01$). Also Ratna et al. (2015) founded negative correlations between such parameters in rice. Yield resulted high related to free phenolic content ($r= 0.594$, $p<0.01$) and inversely correlated with protein content ($r=-0.633$, $p<0.01$) (Table 3.4). It is known as yield reduction that occurred under water stress conditions is generally associated with an increase in protein content (Pompa et al., 2009; Dupont et al., 2006). Regarding the positive correlation between yield and the free phenolic content it was an unexpected result because biotic or abiotic stress were assumed to enhance the phenol concentration as well as to reduce the yield, so a negative correlation was expected. These results are in accordance with another study on sorghum (Sene et al., 2001) but the mechanism remains still unclear. Instead from the ANOVA analysis both species and treatments had a significant impact on yield, while no synergistic effect were highlighted (Table 3.2).

Table 3.1. Yield parameters of millet, sorghum and teff under full and deficit irrigation

	Teff		Sorghum		Millet	
	Full irrigation	**Deficit irrigation**	**Full irrigation**	**Deficit irrigation**	**Full irrigation**	**Deficit irrigation**
Plant height (cm)	211.2±18.7[a]	207.5±14.8[a]	83.43±6.19[b]	68.96±3.69[c]	86.99±2.73[b]	85.58±0.38[b]
1000 kernel weight (g)	0.289±0.008[e]	0.280±0.015[e]	28.98±0.69[a]	27.16±1.18[ab]	4.51±0.56[c]	3.65±0.68[d]
Yield (q/ha)	2.48±0.12e	3.46±0.18[d]	60.04±12.49[a]	22.33±0.93[b]	19.69±1.34[b]	7.41±0.15[c]

*All the data were reported as means ± standard deviation (SD).

**Values with different superscript in the same row are significantly different ($p \leq 0.05$).

3.3.2 Protein and lipid content

Protein and lipid content were not significantly affected by irrigation treatment in all the species investigated, as can be observed from the data in Table 3.2 and Table 3.3. In literature conflicting data on the influence of irrigation treatment on protein and lipid content exists. Queiroz et al. (2013), found an increase in protein content of 100 sorghum genotypes grown without water stress with respect to the same plant cultivated under deficit irrigation. A similar outcome was obtained by Ahmed et al. (2013) in barley. Opposite results were found by Roseberg et al. (2005) in teff grain. Wang & Frei (2011) reviewed the effect of abiotic stress on the quality of agricultural products. They stated that environmental stresses, including drought, usually induce higher protein concentration, with a only few studies showing no effect or lower protein concentration. They explain these results because of the timing of stress occurrence which was an important factor in determining the effect on protein concentration of harvested crops. For example Dwivedi et al. (1996) found that mid-season drought had no effect on the protein concentration of peanut with respect to end-season drought which significantly increased the total protein concentration. In addition the amino acid remobilization as a consequence of a environmental stress leads to stable values of protein content (Rotundo & Westgate, 2009). Also for lipid content it was highlighted that water stress could be ineffective (Bannayan et al., 2008; Palese et al., 2010), in particular with moderate water deficit (Zhu et al., 2015). These facts may partially explain inconsistent effect found in our study on both protein and also lipid content.

Concerning the correlation analysis, proteins were moderately ($p<0.05$) and negatively related with yield (Table 3.4). As previously reported, the negative correlation between yield and protein content is well known in literature (Flagella et al., 2010; Ozturkur & Aydin, 2004). From the ANOVA analysis no effect of irrigation treatment as well as of species on protein content was detected, while a significant effect of species ($p<0.001$) on lipid content was observed. As it is can be seen in Table 3.2, lipid content of the three species investigated was significantly different ranging from 1.39±0.11 in teff to 3.40±0.09 g/100 g d.w. in millet under full irrigation, while lipid content in sorghum was of 2.41±0.22 g/100 g d.w. The same trend was observed in the three species under deficit irrigation too (Table 3.2).

Table 3.2. Protein and lipid content of millet, sorghum and teff under full and deficit irrigation

	Teff		Sorghum		Millet	
	Full irrigation	**Deficit irrigation**	**Full irrigation**	**Deficit irrigation**	**Full irrigation**	**Deficit irrigation**
Protein content (g/100 g d.w.)	16.95±1.17[a]	17.16±0.69[a]	16.96±1.49[a]	18.55±1.73[a]	15.77±0.17[a]	16.53±0.81[a]
Lipid content (g/100 g d.w.)	1.39±0.11[c]	1.58±0.16[c]	2.41±0.22[b]	2.31±0.21[b]	3.40±0.09[a]	3.28±0.08[a]

*All the data were reported as means ± standard deviation (SD).

**Values with different superscript in the same row are significantly different ($p \leq 0.05$).

3.3.3 Phenolic content and total antioxidant capacity

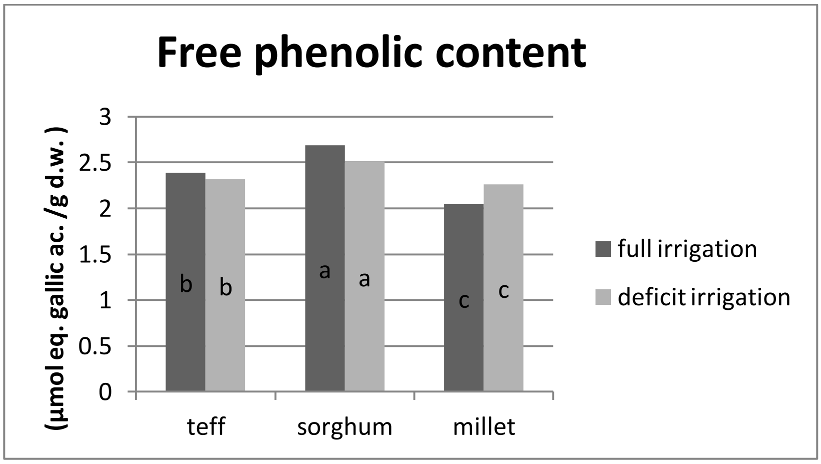
Total antioxidant capacity measured with QUENCHER-ABTS assay was not affected by irrigation treatment, while differences among species were observed (Table 3.3). Teff and sorghum exhibited the highest antioxidant capacity values respect to millet (Figure 3.3).

A similar trend among species was observed in phenolic content of the free fraction (Figure 3.2a), while no effect of deficit irrigation was detected on these parameters (Table 3.3). Most of the studies reported an increase in the antioxidant capacity under deficit irrigation (Stagnari et al., 2014; Ahmed et al., 2013) because of the activation of the phenylpropanoid biosynthetic pathway in response to environmental stress, which lead to an accumulation of phenolics and in turn to an increase in the antioxidant activity. However exception may occur: sometimes a further mechanism could lead to a conversion of phenolic compounds to a more complex phenylpropanoids, such as lignin and other structural polyphenolics, that are not captured in a simple phenolic assay (Wang & Frei, 2011).

A different situation was recorded for bound phenolic content. In fact in this case a strong effect of both species and treatment was observed, as well as of their synergistic effect (Table 3.3). By analyzing the data of bound phenolic assay it is possible to point out an increase of bound phenols only in millet with values of 6.42±0.24 µmol eq. gallic ac. /g d.w. under full irrigation and of 9.92±0.34 µmol eq. gallic ac. /g d.w. under deficit irrigation (Figure 3.2b). Instead in teff a decrease of bound phenolic content was observed from 5.41±0.05 µmol eq. gallic ac. /g d.w under full irrigation to 4.33±0.10 µmol eq. gallic ac. /g d.w under deficit irrigation. It was proposed that normal physiological and biochemical plant processes can be inhibited by severe water deficit (Wu et al., 2017). In addition it was also reported that temperatures above 35°C decrease the concentration of total and individual polyphenols in grains (Wu et al., 2016). In the present study the maximum day-time temperature was, especially in July, always in excess of 35°C (data not shown). So, probably the water stress in combination with high temperatures, might have decreased the biosynthesis of polyphenols in teff.

Figure 3.2. Free (a) and bound (b) phenolic content (μmol eq. gallic ac. /g d.w.) in teff, sorghum and millet under full and deficit irrigation.

(a)



(b)

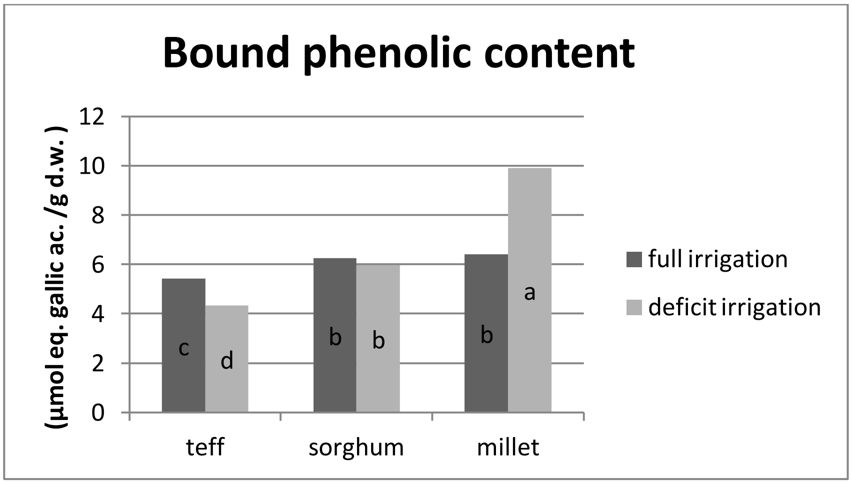
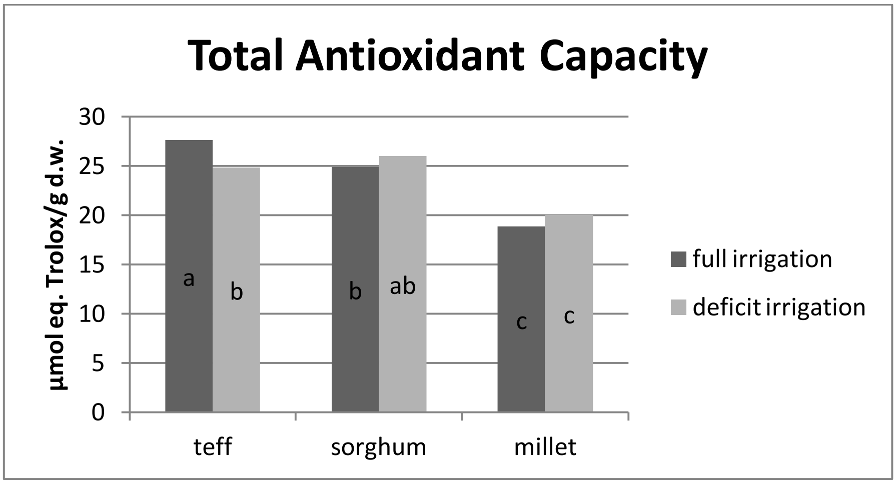


Figure 3.3. Total antioxidant capacity (µmol eq. Trolox/g d.w.) in teff, sorghum and millet under full and deficit irrigation.



The similarities of the effects of species and treatments between free phenolic content and total antioxidant capacity were confirmed by the high correlation between these two parameters, with a r value of 0.600 ($p<0.01$). An interesting and unexpected result was that bound phenolic content is high and negatively correlated with the TAC (Table 3.4). It is generally recognised that in cereal grains bound phenolic compounds are higher respect to the free form, and they strongly contribute to the antioxidant capacity of these plant species (Comert and Gokmen, 2017). Also in our study the bound phenols are higher that free phenols (Figure 3.2), but they clearly do not contribute to the TAC. The assay used to investigate the antioxidant capacity of our samples is an innovative method which do not involve any chemical or enzymatic extraction steps, which are always used in the most common methods for the assessment of the antioxidant activity. The synergistic or antagonistic interactions may partially be ignored due to the different multiple extraction steps. On the contrary the $QHENCHER_{ABTS}$ methods is a simple and direct assay for the antioxidant capacity measurements which was firstly introduced by Serpen et al. (2007) to overcome the problem of the extraction. With this procedure it is possible to determine the antioxidant capacity of a food product by mixing directly the solid samples with the free radicals (Gokmen et al., 2009). In this case all the functional groups of antioxidant compounds can exert their antioxidant capacity. So probably, in our study other compounds besides bound phenols, such as flavonoids, tannins, etc., affected the TAC. For these reasons further investigations on the other functional compounds present in millet, teff and sorghum become necessary to well understand the functional properties of these species.

Table 3.3. Effect of deficit irrigation management, species and their interaction on yield parameters, phenolic content and total antioxidant capacity of all the samples.

Parameters	Irrigation treatment	Species	Irrigation x species
Plant height	0.119	p<0.001	0.184
1000 kernel weight	p<0.05	p<0.001	0.270
Yield	p<0.05	p<0.001	0.057
Protein content	0.153	0.562	0.522
Lipid content	0.740	p<0.001	0.501
Free phenolic content	0.996	p<0.001	0.108
Bound phenolic content	p<0.001	p<0.001	p<0.001
Total antioxidant capacity	0.966	p<0.001	p<0.01

Table 3.4. Correlation coefficient between yield parameters, phenolic content and total antioxidant capacity of all samples.

	Plant height	yield	1000 kernel weight	Lipid content	Protein content	Total antioxidant capacity	Free phenolic content	Bound phenolic content
Plant height	1							
yield	-0.470*	1						
1000 kernel weight	-0.634**	0.713***	1					
Lipid content	-0.780***	0.211 n.s.	0.093 n.s.	1				
Protein content	0.168 n.s.	-0.558*	-0.088 n.s.	-0.333 n.s.	1			
Total antioxidant capacity	0.499*	0.002 n.s.	0.262 n.s.	-0.875***	0.279 n.s.	1		
Free phenolic content	-0.076 n.s.	0.594**	0.633**	-0.321 n.s.	-0.168 n.s.	0.600**	1	
Bound phenolic content	-0.561**	0.011 n.s.	-0.022 n.s.	0.716***	-0.158 n.s.	-0.603**	-0.150	1

Correlation coefficient (r); * P≤0.05, ** P≤0.01, *** P≤0.001, P represents the probability level; n.s. not significant

3.4 Conclusion

In conclusion significant differences among species were observed for all the parameters investigated, except for protein content. In particular sorghum had the highest yield and 1000 kernel weight, millet had the highest lipid content, while teff had the highest antioxidant capacity. Furthermore, deficit irrigation treatment applied in this study showed a significant effect only on yield, 1000 kernel weight and bound phenolic content. Despite the significant effect of deficit irrigation on these parameters, teff yield and 1000 kernel weight didn't significantly change. As a consequence teff appears to be the best-adapted crop to the sub-arid environment of South of Italy due to its best yield stability and nutritional quality also showing a great potential to contribute to food and nutrition security in Mediterranean environments. Further studies will be necessary to better explore under different years and environments the effect of different irrigation treatments on yield and quality performance of the investigated species.

References

AACC International. Approved Methods of Analysis, 11th Ed. Method 30-25.01. Crude Fat in Wheat, Corn, and Soy Flour, Feeds, and Mixed Feeds. Approved November 3, 1999. AACC International, St. Paul, MN, U.S.A.

Abugri DA, Akudago JA, Pritchett G, Russell AE, McElhenney WH (2015) Comparison of Phytochemical Compositions of *Sorghum Bicolor* (L) Moench Red Flour and Pale Brown Leaves. *Journal of Food Science and Nutrition, 1,* 003.

Afify, A. E.-M. M. R., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. A. (2015). Effect of Soaking, Cooking, Germination and Fermentation Processing on Physical Properties and Sensory Evaluation of Sorghum Biscuits. *Notulae Scientia Biologicae; Vol 7, No 1 (2015)DO - 10.15835/nsb719428*.

Afify, A. E.-M. M. R., El-Beltagi, H. S., El-Salam, S. M. A., & Omran, A. A. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine, 2*(3), 203-209. doi: 10.1016/S2221-1691(12)60042-2

Ahmed, I. M., Cao, F., Han, Y., Nadira, U. A., Zhang, G., & Wu, F. (2013). Differential changes in grain ultrastructure, amylase, protein and amino acid profiles between Tibetan wild and cultivated barleys under drought and salinity alone and combined stress. *Food Chemistry, 141*(3), 2743-2750. doi: https://doi.org/10.1016/j.foodchem.2013.05.101

Ahmed, I. M., Nadira, U. A., Bibi, N., Cao, F., He, X., Zhang, G., & Wu, F. (2015). Secondary metabolism and antioxidants are involved in the tolerance to drought and salinity, separately and combined, in Tibetan wild barley. *Environmental and Experimental Botany, 111*, 1-12. doi: https://doi.org/10.1016/j.envexpbot.2014.10.003

Bannayan, M., Nadjafi, F., Azizi, M., Tabrizi, L., & RaStgoo, M. (2008). Yield and seed quality of Plantago ovata and Nigella satiua under different irrigation treatments. *Industrial Crop and Production, 27,* 11–16.

Chandrasekara, A., Naczk, M., & Shahidi, F. (2012). Effect of processing on the antioxidant activity of millet grains. *Food Chemistry, 133*(1), 1-9. doi: https://doi.org/10.1016/j.foodchem.2011.09.043

Cömert, E. D., & Gökmen, V. (2017). Antioxidants Bound to an Insoluble Food Matrix: Their Analysis, Regeneration Behavior, and Physiological Importance. *Comprehensive Reviews in Food Science and Food Safety, 16*(3), 382-399. doi: 10.1111/1541-4337.12263

Coyago-Cruz, E., Corell, M., Moriana, A., Hernanz, D., Benítez-González, A. M., Stinco, C. M., & Meléndez-Martínez, A. J. (2018). Antioxidants (carotenoids and phenolics) profile of cherry tomatoes as influenced by deficit irrigation, ripening and cluster. *Food Chemistry, 240*, 870-884. doi: https://doi.org/10.1016/j.foodchem.2017.08.028

Di Benedetto, N.A., Alfarano, M., Laus, M.N., Caporizzi, R., Soccio, M., Robbe, C., Flagella, Z., Pastore, D. Antioxidant Capacity of Durum Wheat Large Flour Particles May Be Evaluated by QUENCHER ABTS Assay by Adopting a Proper Calculation Mode. *Cereal Research Communications, 43*(4), 682-691

Dlamini, N. R., Taylor, J. R. N., & Rooney, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry, 105*(4), 1412-1419. doi: https://doi.org/10.1016/j.foodchem.2007.05.017

Dupont, F.M., Hurkman, W.J., Vensel, W.H., Tanaka, C., Kothari, K.M., Chung, O.K., Altenbach, S.B. (2006). Protein accumulation and composition in wheat grains: effects of mineral nutrients and high temperature. *European Journal of Agronomy, 25*, 96–107.

Dwivedi, S.L., Nigam, S.N., Rao, R.C.N., Singh, U., & Rao, K.V.S. (1996) Effect of drought on oil, fatty acids and protein contents of groundnut (Arachis hypogaea L.) seeds. *Field Crops Research, 48* (2-3), 125-133.

Farré, I., & Faci, J. M. (2006). Comparative response of maize (Zea mays L.) and sorghum (Sorghum bicolor L. Moench) to deficit irrigation in a Mediterranean environment. *Agricultural Water Management, 83*(1), 135-143. doi: https://doi.org/10.1016/j.agwat.2005.11.001

Flagella, Z., Giuliani, M.M., Giuzio, L., Volpi, C., & Masci, S. (2010). Influence of water deficit on durum wheat storage protein composition and technological quality. *European Journal of Agronomy, 33*, 197–207.

Galindo, A., Collado-González, J., Griñán, I., Corell, M., Centeno, A., Martín-Palomo, M. J., . . . López-Pérez, D. (2017). Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agricultural Water Management*. doi: https://doi.org/10.1016/j.agwat.2017.08.015

Gokmen, V., Serpen, A., Fogliano, V. (2009). Direct measurement of the total antioxidant capacity of foods: the "QUENCHER" approach. *Trends in Food Science and Technology, 20*, 278–88.

Haileselassie, H., Araya, A., Habtu, S., Meles, K. G., Gebru, G., Kisekka, I., . . . Foster, A. J. (2016). Exploring optimal farm resources management strategy for Quncho-teff (Eragrostis tef (Zucc.) Trotter) using AquaCrop model. *Agricultural Water Management, 178*, 148-158. doi: https://doi.org/10.1016/j.agwat.2016.09.002

Inglett, G. E., Chen, D., & Liu, S. X. (2016). Physical Properties of Gluten Free Sugar Cookies Containing Teff and Functional Oat Products. *Journal of Food Research; Vol 5, No 3 (2016)DO - 10.5539/jfr.v5n3p72*.

Lacey, T., & Carol L. (2005). Eragrostis Teff as a Specialised Niche Crop. Government of Western Australia, Department of Agriculture Farmnote. 42

Laus MN, Tozzi D, Soccio M, Fratianni A, Panfili G, Pastore D (2012) Dissection of antioxidant activity of durum wheat (Triticum durum Desf.) grains as evaluated by the new LOX/RNO method. *Jounal of Cereal Science, 56*, 214–222

Laus, M.N., Di Benedetto, N.A., Caporizzi, R., Tozzi, D., Soccio, M., Giuzio, L., De Vita, P., Flagella, Z., Pastore, D. (2015). Evaluation of Phenolic antioxidant capacity in grains of modern and old Durum Weath genotypes by the novel QUENCHERABTS approach. *Plant Foods for Human Nutrition, 70*(2), 207-214

Mansour, E., Abdul-Hamid, M. I., Yasin, M. T., Qabil, N., & Attia, A. (2017). Identifying drought-tolerant genotypes of barley and their responses to various irrigation levels in a Mediterranean environment. *Agricultural Water Management, 194*, 58-67. doi: https://doi.org/10.1016/j.agwat.2017.08.021

Norberg, S., Roseberg, R.J., Charlton, B.A., & Shock, C .(2009). Teff, a New Warm-Season Annual Grass for Oregon. Oregon State University Extension Service, EM 8970-E, Corvallis, OR.

Ozturk, A. & Aydin, F. (2004). Effect of water stress at various growth stages on some quality characteristics of winter wheat. *Journal of Agronomy Crop Science, 190*, 93–99.

Palese, A.M., Nuzzo, V., Favati, F., Pietrafesa, A., Celano, G., & Xiloyannis, C. (2010). Effects of water deficit on the vegetative response, yield and oil quality of olive trees (Olea europaea L., cv Coratina) grown under intensive cultivation. *Scientia Horticolturae, 125*, 222–229.

Pang, B., Zhang, K., Kisekka, I., Bean, S., Zhang, M., & Wang, D. (2018). Evaluating effects of deficit irrigation strategies on grain sorghum attributes and biofuel production. *Journal of Cereal Science, 79*, 13-20. doi: https://doi.org/10.1016/j.jcs.2017.09.002

Pompa, M., Giuliani, M.M., Giuzio, L., Gagliardi, A., Di Fonzo, N., Flagella, Z. (2009) Effect of sulphur fertilization on grain quality and protein composition of Durum Wheat (Triticum durum Desf.). *Italian Journal of Agronomy, 4*, 159–170.

Pontieri, P., Troisi, J., Di Fiore, R., Di Maro, A., Bean, S.R., Tuinstra, M.R., Roemer, E., Boffa, A., Del Giudice, A., Pizzolante, G., Alifano, P., & Del Giudice, L. (2014). Mineral contents in grains of seven food-grade sorghum hybrids grown in a Mediterranean environment. *Australian Journal of Crop Science, 8*(11), 1550-1559

Pradeep, P. M., & Sreerama, Y. N. (2017). Soluble and bound phenolics of two different millet genera and their milled fractions: Comparative evaluation of antioxidant properties and inhibitory effects on starch hydrolysing enzyme activities. *Journal of Functional Foods, 35*, 682-693. doi: https://doi.org/10.1016/j.jff.2017.06.033

Proietti, I., Frazzoli, C., & Mantovani, A. (2015). Exploiting Nutritional Value of Staple Foods in the World's Semi-Arid Areas: Risks, Benefits, Challenges and Opportunities of Sorghum. *Healthcare, 3*(2). doi: 10.3390/healthcare3020172

Queiroz, V. A. V., da Silva, C. S., de Menezes, C. B., Schaffert, R. E., Guimarães, F. F. M., Guimarães, L. J. M., . . . Tardin, F. D. (2015). Nutritional composition of sorghum [sorghum bicolor (L.) Moench] genotypes cultivated without and with water stress. *Journal of Cereal Science, 65*, 103-111. doi: https://doi.org/10.1016/j.jcs.2015.06.018

Ratna, M., Begum, S., Husna, A., Dey, S. R., & Hossain, M. S. (2015). Correlation and path coefficients analyses in Basmati rice. *Bangladesh Journal of Agricultural Research; Vol 40, No 1 (2015)*.

Roseberg, R.J., Norberg, S., Smith, J., Charlton, B., Rykbost, K., & Shock, C. (2005). Yield and quality of teff forage as a function of varying rates of applied irrigation and nitrogen. *Annual report*, 119- 136.

Rotundo, J.L., & Westgate, M.E. (2009). Meta-analysis of environmental effects on soybean seed composition. *Field Crop Research, 110,* 147–156.

Samarah, N.H. (2005). Effects of drought stress on growth and yield of barley. *Agronomy of Sustainable Development*, 145–149.

Seghatoleslami, M.J., Kafi, M., & Majidi, E. (2008). Effect of deficit irrigation on yield, WUE, and some morphological and phenological traits of three millet species. *Pakistan Journal of Botany, 40*(4), 1555-1560.

Sene, M., Dore, T., & Gallet, C. (2001). Relationship between biomass and phenolic production in grain sorghum grown under different conditions. *Agronomy Journal, 93*, 49-54.

Serpen, A., Capuano, E., Fogliano, V., & Gokmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry, 55,* 7676–7681.

Serpen, A., Gokmen, V., Pellegrini, N., Fogliano, V. (2008). Direct measurement of the total antioxidant capacity of cereal products. *Journal of Cereal Science, 48*, 816-820

Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods, 5*(2), 570-581. doi: https://doi.org/10.1016/j.jff.2013.02.004

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol 299*, 152–178.

Stagnari, F., Galieni, A., Speca, S., & Pisante, M. (2014). Water stress effects on growth, yield and quality traits of red beet. *Scientia Horticulturae, 165*, 13-22. doi: https://doi.org/10.1016/j.scienta.2013.10.026

Taylor, J. R. N., Belton, P. S., Beta, T., & Duodu, K. G. (2014). Increasing the utilisation of sorghum, millets and pseudocereals: Developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science, 59*(3), 257-275. doi: https://doi.org/10.1016/j.jcs.2013.10.009

Yau, S.K., Nimah, M., Farran, M. (2011). Early sowing and irrigation to increasebarley yields and water use efficiency in Mediterranean conditions. *Agricultural Water Management, 98*, 1776–1781.

Yihun, Y.M., Haile, A.M., Schultz, B., & Erkossa, T. (2013). Crop water productivity of irrigated teff in a water stressed region. Water resources management, 27, 3115-3125. Doi: 10.1007/s11269-013-0336-x

Wang, Y., & Frei, M. (2011). Stressed food – The impact of abiotic environmental stresses on crop quality. *Agriculture, Ecosystems & Environment, 141*(3), 271-286. doi: https://doi.org/10.1016/j.agee.2011.03.017

Wu, G., Johnson, S., Bornman, J., Bennett, S., Singh, V., & Fang, Z. (2016a. Effect of genotype and growth temperature on sorghum grain physical characteristics, polyphenol content and antioxidant activity. *Cereal Chemistry, 93*, 4

Wu, G., Bennett, S. J., Bornman, J. F., Clarke, M. W., Fang, Z., & Johnson, S. K. (2017). Phenolic profile and content of sorghum grains under different irrigation managements. *Food Research International, 97*, 347-355. doi: https://doi.org/10.1016/j.foodres.2017.04.030

Wu, G., Johnson, S. K., Bornman, J. F., Bennett, S. J., & Fang, Z. (2017). Changes in whole grain polyphenols and antioxidant activity of six sorghum genotypes under different

irrigation treatments. *Food Chemistry, 214*, 199-207. doi: https://doi.org/10.1016/j.foodchem.2016.07.089
Zhu, F. (2018). Chemical composition and food uses of teff (Eragrostis tef). *Food Chemistry, 239*, 402-415. doi: https://doi.org/10.1016/j.foodchem.2017.06.101
Zhu, Y., Taylor, C., Sommer, K., Wilkinson, K., & Wirthensohn, M. (2015). Influence of deficit irrigation strategies on fatty acid and tocopherol concentration of almond (Prunus dulcis). *Food Chemistry, 173*, 821-826. doi: https://doi.org/10.1016/j.foodchem.2014.10.108

CHAPTER 4. The use of teff flour (*Eragrostis tef*) in extruded cereal breakfast and the effects on physical and antioxidant properties

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Abstract

Teff is a minor cereal crop with an excellent nutritional value so its use is interesting for obtaining healthy products. This study aims to investigate the addition of teff flour in a mass ratios of 30, 50 and 70 % to rice flour, and the effects of two process parameters, feed moisture (varied from 16 to 18%) and temperature (from 100 to 140°C), on the quality of innovative extruded cereal breakfast. Sectional Expansion Index was affected by feed moisture and temperature, with negative (-3.34) and positive (2.80) effects, respectively. Lightness decreased with addition of 70% of teff flour. Crispness was negatively influenced by increasing teff flour, while the crispiest products was obtained at 120°C. Hardness varies considerably from 35.785±3.525 to 60.302±6.151 by increasing temperature and with low feed moisture. Moreover high temperature of 140°C and 70% of teff flour led to smaller pores. Total Antioxidant Capacity was influenced by only teff flour.

Teff flour could be useful for developing healthy extruded cereal breakfast with physical and microstructural properties fitting the consumers' acceptance, optimizing the process conditions.

Keywords: teff; physical properties; microstructural properties; extrusion technology; desirability approach; total antioxidant capacity

4.1 Introduction

The demand for gluten-free foods is certainly increasing in the last years. Among the alternative grains with gluten-free nature, teff is a suitable food for celiac patients and/or these suffering from gluten-sensitivity. Teff (*Eragrostis tef* (Zuccagni) Trotter) is a tropical cereal crop indigenous to Ethiopia that grows in a wider ecology. Literature greatly highlighted his attractive nutrient profile. Its starch content of ~73% is higher than in other cereals making teff a potential good sources of food energy in gluten free products (Gebremariam, Zarnkow, & Becker, 2014). Furthermore, it exhibits an excellent balance among the essential amino acids whose content is comparable to the egg, except for lysine and isoleucine, even though lysine content is higher than to other cereals (Gebremariam et al., 2014). Finally teff shows a high content in fibre and bioactive compounds (Zhu, 2018) being a potential ingredient to satisfy the increasing demand of functional foods. Despite above consideration, teff is rarely used in food industry and only limited research on its potential role in food products have been performed in detail. In fact the processing techniques and the type of food products manufactured from teff remain traditional; in Ethiopia it is mostly used as a staple food by making flat bread, known as *Injera*. Recently, however, some studies were focused on the development of teff-based products, like bread (Alaunyte, Stojceska, Plunkett, Ainsworth, & Derbyshire, 2012; Marti et al., 2017), cookies (Inglett, Chen, & Liu, 2016), muffin (Valcarcel, Ghatak, Bhaduri, & Navder, 2012) and breakfast cereal (Solomon, 2014).

Currently ready-to-eat (RTE) breakfast cereals are widely used in the daily life because of their convenience, cheapness and because they are frequently fortified with some micronutrients or fibre, making them more attractive for health conscious consumers (Albertson, Anderson, Crockett, & Goebel, 2003). The most popular breakfast cereals, second only after muesli or conventional flakes, are expanded products, especially in the Western countries (Wójtowicz et al., 2015).

Extrusion cooking is the most important technology used to produce RTE breakfast cereals, flat breads, snacks, etc. It is a versatile, short-time and low-cost technology used by food industries (Oliveira, Rosell, & Steel, 2015). In the extrusion cooking process, a drop of pressure after die causes immediate evaporation of water embedded in the fluid and the formation of pores leading to a wide expansion. Structure of extrudates can be significantly determined by the ingredient used and by the extrusion process variables, among which temperature profile of the extruder, water content of the mass and screw speed are very important. These parameters affect the distinct properties of the end-product, such as physical (sectional expansion, porosity, wall thickness, etc.), sensory (crispiness, hardness, etc.) and functional (antioxidant capacity, phenolic compounds, fibre content, etc.) characteristics. For instance most of the studies carried out in the extrusion process developing functional snacks or breakfast cereals involved whole wheat flour (Oliveira et al., 2015; Wójtowicz et al., 2015), whole grain sorghum (Mkandawire, Weier, Weller, Jackson, & Rose, 2015), oat flour (Holguín-Acuña et al., 2008), or enriched cereals-based products with innovative functional ingredients, such as guar or inulin (Brennan, Monro, & Brennan, 2008), blackcurrant juice (Mäkilä et al., 2014), lycopene (Dehghan-Shoar, Hardacre, & Brennan, 2010), fruit powders (Potter, Stojceska, & Plunkett, 2013), etc. In only few cases the use of teff flour for extruded products was taken into account (Solomon, 2014; Stojceska, Ainsworth, Plunkett, & İbanoğlu, 2010). Therefore there is a lack of available information regarding the physical and structural properties of teff-based cereal breakfast.

This paper is dedicated to the introduction of teff flour in ready-to-eat breakfast cereals. More specifically the effects of teff flour addition, feed moisture and temperature were studied in order to improve our knowledge on the use of this cereal to obtain innovative breakfast products.

4.2 Material and methods

4.2.1 Raw materials

Rice and teff flours were supplied by Molino Maraldi sas (Cesena, Italy) and stored at 4°C until use. Table 4.1 summarized the proximate composition of the flours as reported from the producers.

Table 4.1 Proximate composition of raw materials

Raw materials	Moisture (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Fiber (%)
Teff flour	15.5	2.0	11.8	62.7	7.6
Rice flour	14.0	0.5	7.3	87.0	1.0

4.2.2 Sample preparation and extrusion experiments

Three different blends of flours were prepared by using mass ratios between teff and rice flours of 30:70, 50:50, 70:30. The samples were packed in polyethylene bags and stored at room temperature for a maximum of 24 h before extrusion experiments.

RTE cereal breakfasts were obtained by using a co-rotating twin-screw extruder BC-21 CLEXTRAL (Firminy, France). Temperature of the last barrel zone (°C), feed moisture of the blend (%) and flour blend compositions, by modulating the mass ration of teff flour (%), were analysed in this study as independent variables. In order to define the suitable extruder operating conditions and the raw materials levels, some preliminary trials were performed.

Then, a Box-Behnken design was used to study the effect of each independent variable on some quality indices of the snacks.

The total working barrel length (L) and the screw diameter (D) were 900 mm and 25 mm, respectively, with a L/D of 36:1, while the circular die diameter was of 45 mm. The temperature profile of the first 6 barrel sections was of 40-50-60-70-80-90-95 °C for all the experiments, while three different temperatures of 100, 120 and 140 °C were used in the last barrel section. The water feed rate was kept constant at 10.5 mL/min and screw speed was of 300 rpm, while feed rate was varied from 10.44 to 18.81 kg/h in order to modify the feed moisture of the blends from 16 to 18%. The coded and the real values of the independent variables are shown in Table 4.2.

Once the extrusion conditions were constant, samples of the extrudates were cut with a sharp knife of approximately 20 cm long, as they emerged from the die, to be used for physical and structural analysis. All samples were left to equilibrated at room temperature for 30 min before to be dried at 60 °C in a forced-air convection oven to ~6 % moisture in order to avoid the effect of moisture on the chemical and physical analyses. Then they were packed into polyethylene bags and stored at room temperature.

4.2.3 Physical analysis

4.2.3.1. Moisture content and moisture loss

Moisture determination of raw products and extruded samples were conducted according to the AOAC methodology (2000) in triplicates.

Table 4.2 Box-Behnken design for extruded cereal breakfast

RUN	CODED VALUES			REAL VALUES		
	X_1	X_2	X_3	**Teff flour (%) (X_1)**	**Temperature (°C) (X_2)**	**Feed moisture (%) (X_3)**
1	1	-1	0	70	100	17
2	-1	-1	0	30	100	17
3	1	1	0	70	140	17
4	-1	1	0	30	140	17
5	1	0	-1	70	120	16
6	-1	0	-1	30	120	16
7	1	0	1	70	120	18
8	-1	0	1	30	120	18
9	0	-1	-1	50	100	16
10	0	1	-1	50	140	16
11	0	-1	1	50	100	18
12	0	1	1	50	140	18
13	0	0	0	50	120	17
14	0	0	0	50	120	17
15	0	0	0	50	120	17

4.2.3.2 Sectional Expansion Index (SEI)

The SEI was calculated as the ratio between the diameter of the extruded product (Dc) and the diameter of the die (Dm) (Brennan et al., 2008). Ten pieces from each trial run were randomly sampled and the diameter was measured in two different parts of the extrudate with a universal Crafsman caliper.

4.2.3.3 Determination of color parameters

The raw flours and the extruded samples were analyzed for color readings. The extruded products were previously milled using a laboratory grinder GRINDOMIX GM-200 (Retsch GmbH, Germany) for 30 seconds at 4000 rpm to obtain a fine powder which was packed into a shallow dish. Color readings were taken from five separate points on the surface of the powders using a MINOLTA Chroma Meter (CR-400, Konica Minolta, Inc., Japan). The values of luminance (L*), red index (*a**) and yellow index (*b**) were recorded (Dehghan-Shoar et al., 2010). Five replicates for each sample were performed.

4.2.3.4 Instrumental texture

Texture profile analysis was performed using a texture analyzer mod. TA-XTplus (Stable Micro Systems, Surrey, UK). An aluminum cylinder of 35 cm diameter (P/35; Stable Micro Systems, Surrey, UK) was used and the test speed was adjusted to 1 mm s^{-1}. A single cereal sample was axially compressed to 70% of the original height with 5 g trigger force. Also, a pre-test speed of 1.5 mm/sec, test speed of 1 mm/sec and post-test speed of 10 mm/sec, were used during analysis. The force required to compress the sample at 70% was recorded as the Hardness of the cereal product, as reported by Brennan et al. (2008). The numbers of peaks recorded during the 70% compression was recorded as the Crispiness of the product. All results were analyzed using the software EXPONENT version 2.0.6.0 (Stable Micro System, Surrey, UK). The results represented the average of ten measurements. In order to guarantee the homogeneity of the analysis 1.5 mm of each sample were sliced.

4.2.4 X-ray computed microtomography analysis

Microtomographic images of the samples were obtained by using a SkyScan 1174 micro-CT scanner (Brüker, Kontich, Belgium). The scanning was performed over 180°, with rotation steps of 0.5° and an averaging frame of 2, obtaining 720 projections.

Reconstructed cross-section images were obtained by using Nrecon 1.6.2.0 (Bruker, Kontich, Belgium). 2D and 3D analyses of the samples were performed by using CTAn 1.12.0.0 (Bruker microCT, Belgium). Image processing consisted in the image thresholding, performed by using Otsu's method, the definition of a modular region of interest (ROI), obtained by using the function shrink-wrap, which automatically wraps the original ROI around the boundaries of the object. The main microstructure properties were evaluated on the entire object consisting of total cross-sectional images of N=350. All measurements were performed in triplicate.

4.2.5 Total Antioxidant Capacity (TAC)

The direct $\text{QUENCHER}_{\text{ABTS}}$ assay for the evaluation of TAC was applied as reported by Serpen et al. (2008) and modified by Di Benedetto et al. (2015) and Laus et al. (2015). The analysis were performed in triplicates on each samples.

4.2.6 Statistical analysis

The effects of each independent variable on quality indexes were determined by ANOVA and Fisher test with a significance of 0.05. Moreover, experimental data were fitted by the polynomial model of Eq.1 with the aim to evaluate linear, non-linear and interactive effect of independent variables.

$y = B_0 + \sum B_i \times_i + \sum B_{ii}\, x^2{}_{ii} + \sum B_{ij} \times_{ij}$ (Eq.1)

Where, y is the response variable; B_0, B_i, B_{ii}, B_{ij}, are the regression coefficients for constant, linear, quadratic and interaction regression; x_i, are the coded values for linear effects of the independent variables; x^2 are the coded values for quadratic effects of the independent variables; $\times_{ij}$ are the coded values for interaction effects of the independent variables. Statistical analysis was performed using Statistica ver. 10 software (Statsoft, Tulsa, USA).

In addition the optimization of multiple responses was performed by using the desirability function proposed by Derringer and Suich (1980) and as reported by Derossi et al. (2015).

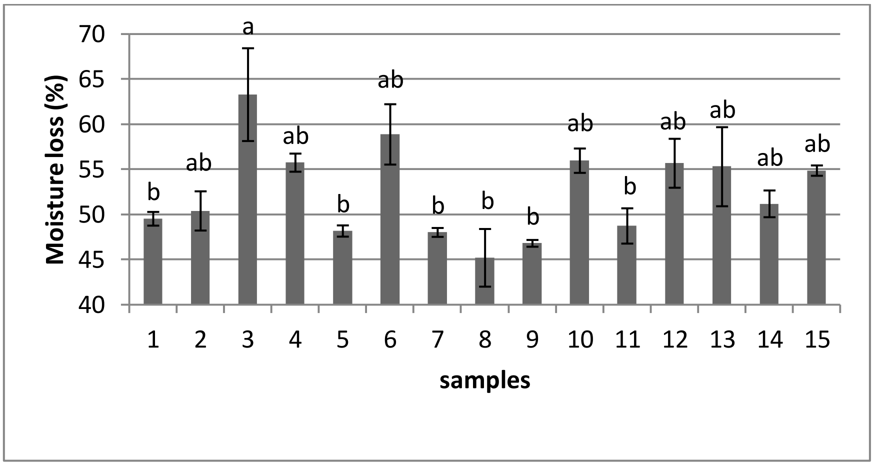
4.3. Results and Discussion

4.3.1 Moisture content and moisture loss

Mass fraction of water of the extruded products varied between 6.24±0.67 % and 9.86±0.93 % within all treatments (data not shown). The greatest fractional decrease in water content was of 63 % for sample 3 extruded at the highest temperature of 140°C and with the highest teff flour enrichment of 70 % (Figure 4.1).

Probably the high temperature of the extrusion process improved the moisture loss of the dough. Moreover, the fiber content of teff flour higher than the rice flour, may lead to a weaker water holding capacity. Any differences among all samples prepared by changing moisture content were not observed.

Figure 4.1 Moisture loss (%) in extruded cereal breakfast samples

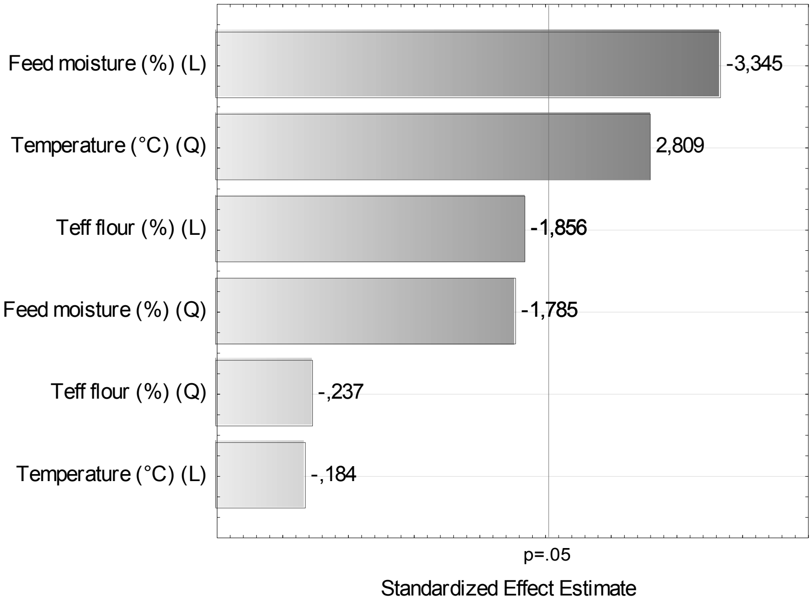


Data shown are means of 3 experiments. Values with the same letters are not significantly (p<0.05) different from each other

4.3.2 Sectional Expansion Index (SEI)

Expansion is an important physical attribute for the extruded products that greatly affects consumer acceptability because of its correlation with hardness and crispness, which are perceptive attributes to consumers (Oliveira, Schmiele, & Steel, 2017). Figure 4.2 shows the effects of independent variables on the SEI of extruded breakfast cereals. The feed moisture showed a linear relationship with SEI with an estimated effect of -3.34 ($p<0.05$), while temperature showed a non-linear (quadratic) relation with an estimated effect of 2.80. Several authors reported a prominent effect of moisture on SEI respect to temperature (Oliveira et al., 2017; Wójtowicz et al., 2015), which was in accordance with our results. It was detected that the expansion ratio mainly depends on feed moisture: the higher moisture was applied, the lower expansion of extrudates was observed. This is probably due to the elastic force that are dominant at low moisture content (Ding, Ainsworth, Tucker, & Marson, 2005). Regarding temperature, the maximum value of SEI was observed at 120°C, while it tends to decrease at 140°C (data not shown). Moraru and Kokini (2003) showed that the expansion of extruded products was enhanced by increasing temperature until an optimum point after that it newly decreased. It is recognized that extrusion temperature plays an important role in changing the rheological properties of the extruded melt, which in turn affects the expansion volume. Depending on the type of starch, there is a temperature range in which the expansion of starch reaches the maximum. After this critical temperature, the expansion decreases most likely due to the degradation of the starch melt, which finally collapse. It can be hypothesized that a temperature of 120°C was the optimum point for the expansion of our products made with rice and teff flour, while reaching 140°C the starch melt was partially degraded. Finally, teff flour not significantly influences SEI; this means that enriching rice-based extruded cereal breakfasts with teff flour, the obtained products could keep dimensional properties of the most common extruded products.

Figure 4.2 Estimated Effects of independent variables on Sectional Expansion Index (SEI) of extruded samples



4.3.3 Instrumental color

The estimated effects of the independent variables on the color parameters are shown in Table 4.3. L* values were significantly influenced ($p<0.05$) by teff flour, with a negative and linear effect of -7.05, and by feed moisture, with a non linear and positive effect of 2.15. Figure 4.3 shows the 3D plot, describing the effect of these two variables on the lightness of extruded samples. According to Table 4.3, a linear increase of L* was observed as a function of the mass fraction of teff flour with the highest L* value of 77.87±1.30 for samples at 30% of teff flour and 17% of feed moisture. This was expected since the high fiber content gives a brown aspect to the flour as also reported by several authors (Holguín-

Acuña et al., 2008; Oliveira et al., 2017). Lightness was also affected by feed moisture which, when increased from 16% to 17%, led to an increase of L* value reaching a maximum value of ~77 at 17% of moisture and 30% of teff flour, after that it slightly decreased until ~75.

Redness (a*) was affected by all the independent variables investigated in this study with linear estimated effects of 7.15, 3.12 and 2.35 for teff flour, feed moisture and temperature, respectively.

Regarding the effect of temperature, our results were in agreement with several studies which reported an increase of redness with high temperature and this was probably due to the increasing rate of Maillard browning reaction due to the high barrel temperature which increased the total color change (Ilo, Liu, & Berghofer, 1999; Mäkilä et al., 2014; Oliveira et al., 2017).

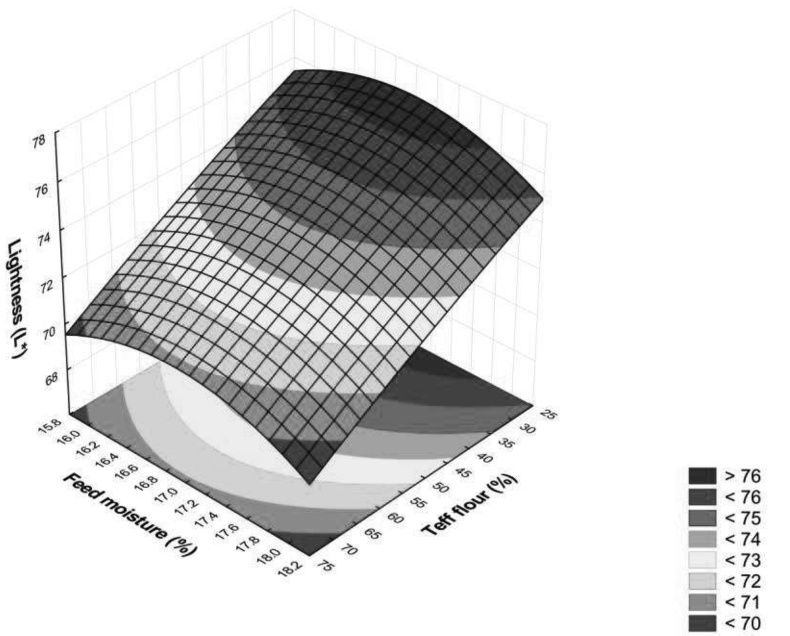
Yellowness (b*) was slightly influenced by teff flour and strongly affected by feed moisture showing estimated effect of 2.22 and 6.66, respectively. Overall, b* values (data not shown) increased after the extrusion process. However, as well known, during the extrusion process, the color changes may be attributed to many reactions including Maillard reaction, caramelization, hydrolysis and pigment degradation (Santillán-Moreno, Martínez-Bustos, Castaño-Tostado, & Amaya-Llano, 2011). Probably the increment of b* values was the result of these different reactions which synergically influence this parameter.

Table 4.3 Estimated Effects of the independent variables on color indices of extruded samples

Independent variables	Estimated effects		
	Lightness (L*)	**Red index (a*)**	**Yellow index (b*)**
Teff flour (%) (L)	-7.05*	7.15*	2.52*
Teff flour (%) (Q)	-0.27	0.33	0.14
Feed moisture (%) (L)	-0.58	3.12*	6.66*
Feed moisture (%) (Q)	2.15*	0.27	-0.72
Temperature (°C) (L)	-0.58	2.35*	-0.80
Temperature (°C) (Q)	-1.93	0.95	1.12

*Statistically significant at $p<0.05$
L: linear effect
Q: quadratic effect

Figure 4.3 Surface response plot describing the effects of teff flour (%) and feed moisture (%) on the Lightness (L*) of extruded samples

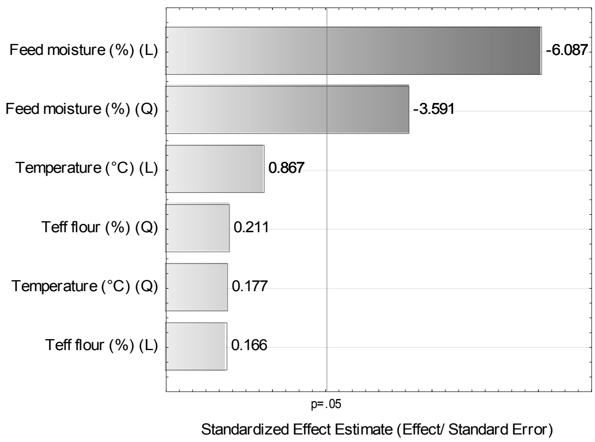


4.3.4 Instrumental texture

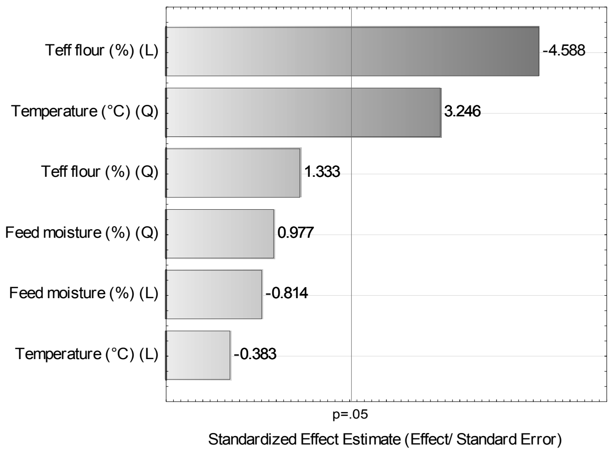
Figure 4.4 show the Pareto chart describing the effect of independent variables on the hardness (Figure 4.4a) and crispness (Figure 4.4b) of extruded samples, respectively. Hardness was mainly affected by feed moisture by both linear and non linear effects of -6.08 and -3.59 respectively. This indicates an increase of hardness when decreasing feed moisture. On the other hand, the crispness of samples was mainly related with the mass fraction of teff flour with an estimated linear effect of -4.58, while temperature exhibited a non linear and positive effect of 3.24.

Figure 4.4 Effects of independent variables on Hardness (a) and Crispiness (b) of extruded samples

a)



b)



According to statistical results, Figure 4.5 shows as the samples obtained at the lowest feed moisture were the hardest, while samples extruded at higher feed moisture were the most brittle. This was probably due to the retrogradation of starch. Jung et al. (2017) asserted that the degree of retrogradation in rice is inversely related to moisture content, and the retrogradation of gelatinized rice resulted in an increase of hardness. Probably in our samples the decrease in feed moisture led to an increase of retrograded starch during cooling and therefore to an increase in hardness. In this study temperature was not a significant variable influencing hardness of the products. Generally the tendency to increase hardness at higher temperature may be due to the higher melt temperature that may cause changes in the chemistry of the melt (Dehghan-Shoar et al., 2010). But this is right for temperature higher than 180°C which are no reached in this study.
Regarding the effect of teff flour enrichment, our result did not show any effect of the hardness of samples. This could be unexpected because of the fiber content by substituting starch reduces the proportion of materials capable to form a melt in the extruder and this could influence the final hardness. However, also Valcarcel et al. (2012), who investigated the physical, textural and sensory properties of muffin prepared with teff flour, proved that a substitution till to 50% of rice flour with teff flour did not significantly affect the textural characteristics of the muffins.

Figure 4.5 Surface response plot describing the effects of feed moisture (%) and teff flour (%) on Hardness (N) of extruded samples

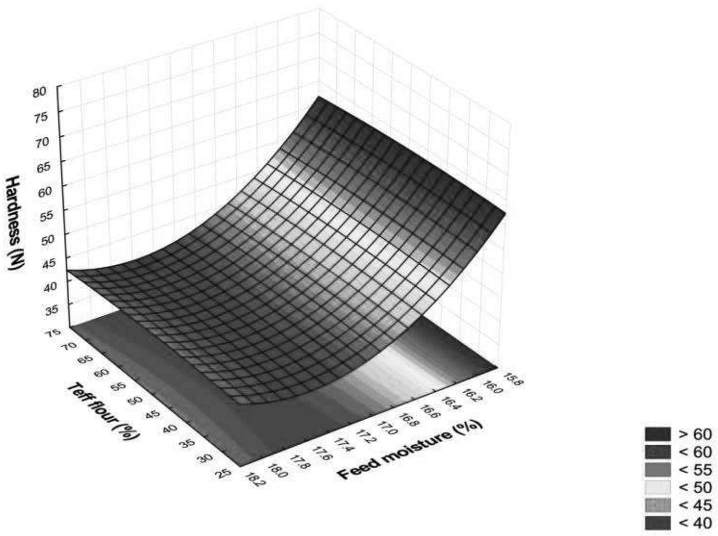
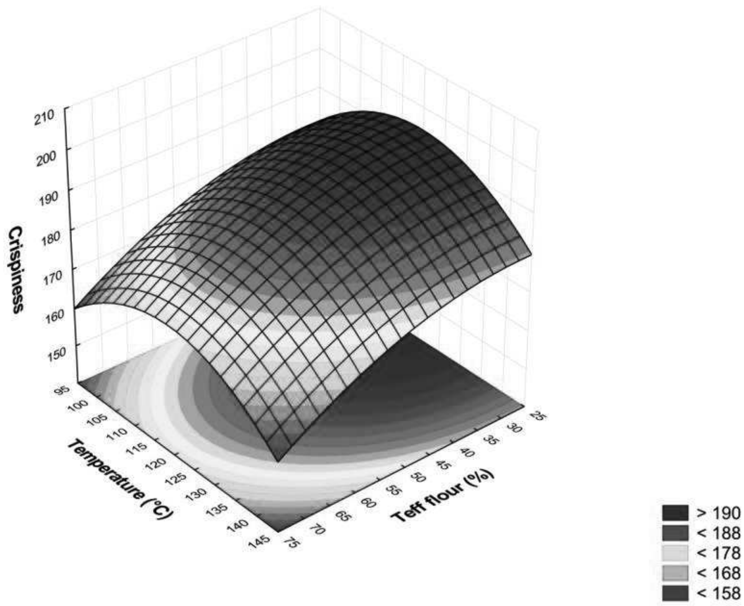


Figure 4.6 reports the 3D plot describing the effect of teff flour enrichment and extrusion temperature on the crispness of the samples. Increasing teff flour from 30 to 70% at a temperature of 100°C, crispness decreased from 190.323±9.884 to 162.500±12.930. Comparable differences in crispness were highlighted increasing teff flour at 120 and 140°C. Similarly, Oliveira et al. (2017) founded a negative effect of whole grain extruded snacks on crispness. This is probably due to the presence of insoluble fibers, which reduced elasticity, and the weakening of cell structure when starch-fiber interactions take places (Robin et al., 2012). Indeed, a slight increase of temperature until 120°C formed a softer and crispy extrudates, although a further increase produced a decrease in the crispness.

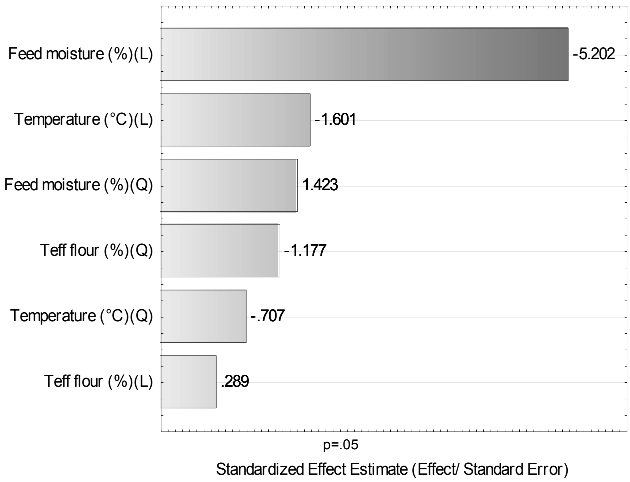
Figure 4.6 Surface response plot describing the effects of temperature (°C) and teff flour (%) on Crispness of extruded samples



4.3.5 Microstructural properties of extruded samples

Pareto chart describing the effect of independent variables on the total porosity of the extruded samples (Figure 4.7) shows as feed moisture had a linear and negative estimated effect of -5.20, while no significant effects were highlighted for the other variables. These results were in accordance with the effect of feed moisture on SEI (Figure 4.2). In fact by decreasing feed moisture from 18 to 16%, the expansion of the sample increased leading to an increase in porosity fraction. Probably the higher porosity of the low feed moisture extrudates indicates that starch granules were less disrupted and tended to cause a high expansion (Alam et al., 2014).

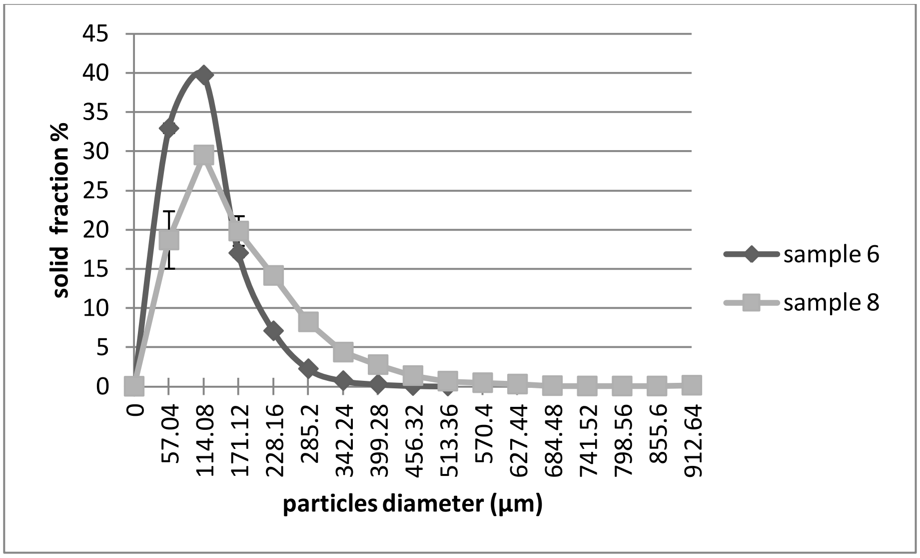
Figure 4.7 Effect of independent variables on total porosity (%) of extruded samples



To better understand the effect of the independent variables on the microstructural properties of the extruded samples the size distribution functions of both solid phase and of the voids of the extruded samples were analysed.

Figure 4.8 shows the size distribution function of solid phase of extruded snack at two different moisture contents of 16% and 18%. When the lower feed moisture was used (sample 6), a peak of ~40% was observed at 114.08 µm indicating as the majority of the wall thickness was small in size, while any solid elements bigger than 399.28 µm were not observed. On the other hand by increasing feed moisture at 18% (sample 8), the solid phase was characterized by less solid elements having a size of 114.08 µm which exhibited a volume fraction of ~30%. Moreover after this peak, the size distribution function decreased slower, reaching a maximum of solid particles diameter of 912.64 µm and indicating that the solid elements were bigger in size. These data were in agreement with the above-discussed results. At low feed moisture a higher expansion and smaller wall thickness were observed. On the contrary high feed moisture led to a minor expansion of samples and a greater size of the solid elements of extrudates. According to these results, Saeleaw, Dürrschmid, and Schleining (2012) reported that high feed moisture extruded snacks result in thicker cell walls.

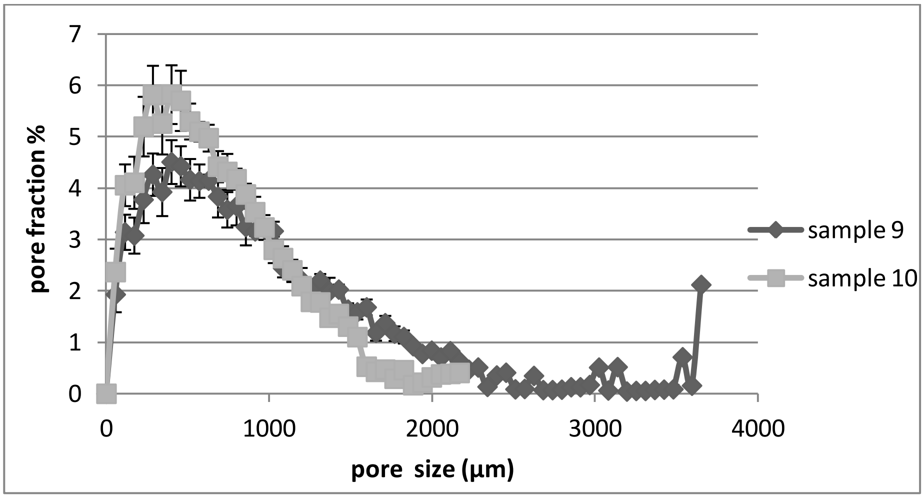
Figure 4.8 Size distribution function of the solid phase of extruded samples at different feed moisture



The data shown are the mean values of three independent replicates. The *error bars* indicate the standard deviation

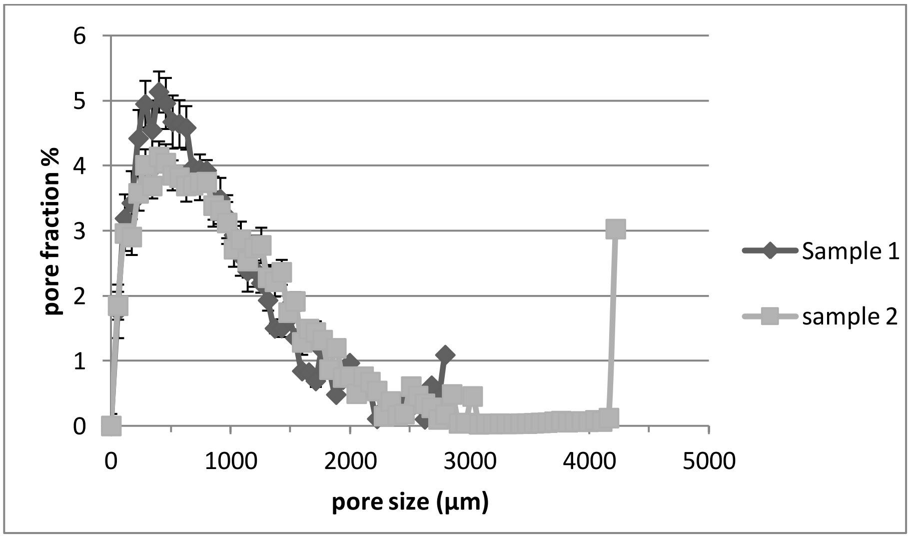
Regarding the pore size of the samples, it was significantly affected by varying both temperature and teff flour ($p<0.05$). As examples, Figure 4.9 shows the distribution of voids of samples 9 and 10, extruded at 100°C and 140°C, respectively, keeping constant all other conditions. From the cumulative distribution it was calculated that, for sample 9, a void fraction of ~62% is characterized by pores having a diameter <1000 µm while for sample 10 a greater volume fraction of ~77% was occupied by these kind of pores. Moreover, in this case, any pore with a diameter greater than 2167 µm was not observed, while sample 9 showed a volume fraction of 7.5% with pores larger than 2167 µm. Probably at higher temperature the abruptly change in pressure and temperature at the die favored a more homogeneous water evaporation leading to the formation of more voids. On the other hand at temperature of 100°C few big pores were produced at the die of the extruder.

Figure 4.9 Size distribution function of the voids of extruded samples at different temperature



The data shown are mean values from three independent replicates and the *error bars* indicate the standard deviations.

Figure 4.10 Size distribution function of the voids of extruded samples at different teff flour mass ratio

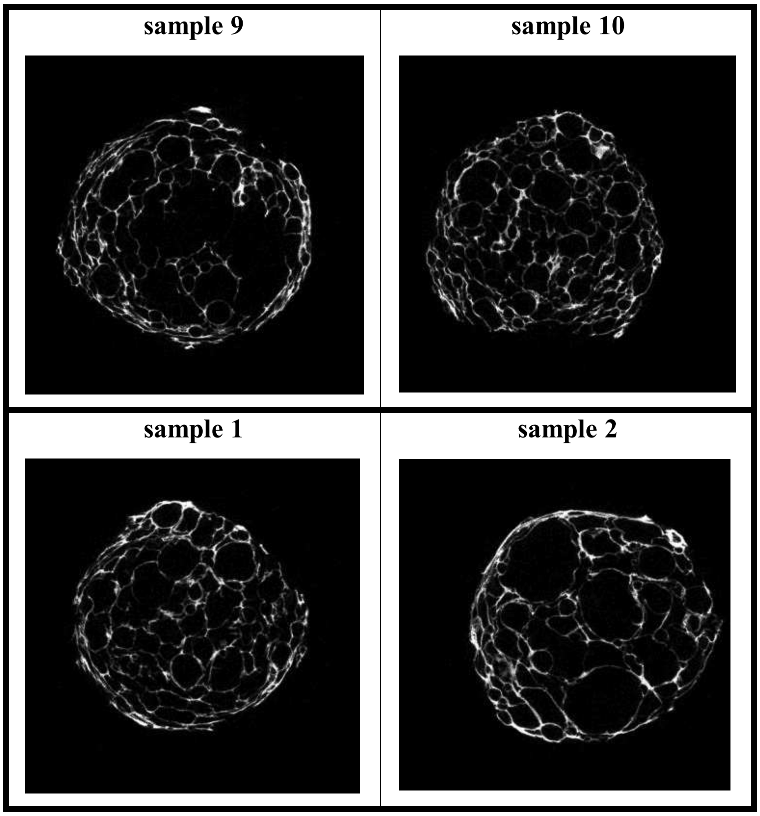


The data shown are the media of three independent replicates and the *error bars* indicate the standard deviations.

Figure 4.10 shows the size distribution function of pores for extrudates obtained with 30 and 70% of teff flour. In general as the content of teff flour increased as the peak of distribution function became sharper with slighter tails. From the cumulative distribution it was calculated that pores having a diameter less than 1000 μm occupied a volume fraction of 68% and 59% for samples containing the 70% and 30% of teff flour, respectively.
Furthermore, for extrudates containing 70% of teff flour no pores with a diameter ≥ 3000 μm were observed, while the samples with 30% of teff flour exhibited pores with a diameter greater than 3000 μm even though they occupied around the 5% of the total void phase.

Figure 4.11 shows some representative cross-sectional microtomographic images of the extruded samples obtained in different experimental conditions. According to the pore size distribution function, the samples obtained at the highest temperature showed the greatest number of small pores, while at 100°C the existence of bigger pores was clearly observed. Also from the figure is possible to observe that sample 2 is characterized from few pores bigger in size, while sample 1 showed copious small pores. Some studies suggest that the addition of protein and fiber may increase nucleation sites in the melt, thereby forming smaller pores as water vaporizes at the die (Devi et al., 2013; Ramos Diaz et al., 2013; Ramos Diaz et al., 2015). This might explain the higher number of smaller pores for samples enriched with teff flour. Moreover it is believed that the size of pores played a fundamental role in the perception of the crispiness (Ramos Diaz et al., 2015). As expected, extrudates with 70% of teff flour, which correspond to a greater number of small pores, were inversely related to crispiness, as reported in Figure 4.6. This means that a high crispiness corresponds to a huge amount of high pores of extrudates.

Figure 4.11 Representative cross sectional images of breakfast samples extruded under different experimental conditions.



4.3.6 TAC of extruded samples

The only independent variable which had a significant effect on the TAC of extruded samples were teff flour, with linear and non linear values of 7.794 and -4.769, respectively (Figure 4.12). These results were expected because of the recognized high antioxidant capacity of teff flour (Collar et al., 2014; Forsido et al., 2013), even if no studies were available on the TAC of extruded teff flour with $\text{QUENCHER}_{\text{ABTS}}$ approach. The highest TAC highlighted in extruded samples with 70% of teff flour respect to samples with 30% of teff flour is probably due to the high phenolic and fibre content, which both contribute to the total antioxidant capacity (Gebremariam et al., 2014; Guo & Beta, 2013). On the other hand neither temperature nor feed moisture had a significant impact on extruded samples.

Figure 4.12 Effect of independent variables on TAC (µmol eq. Trolox/ g d.w.) of extruded samples

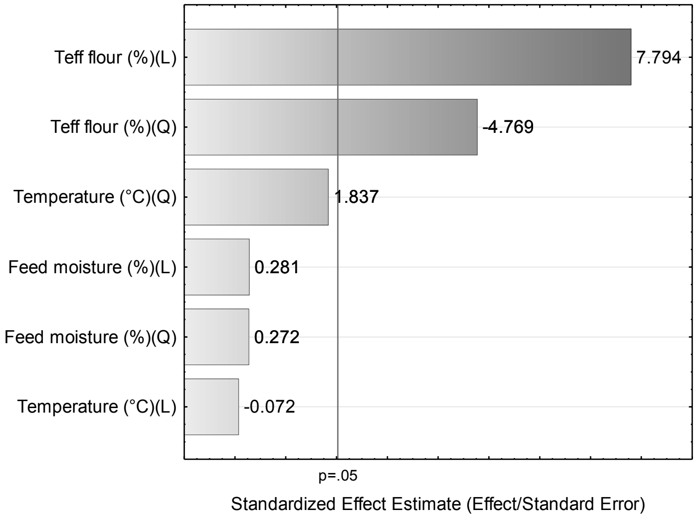
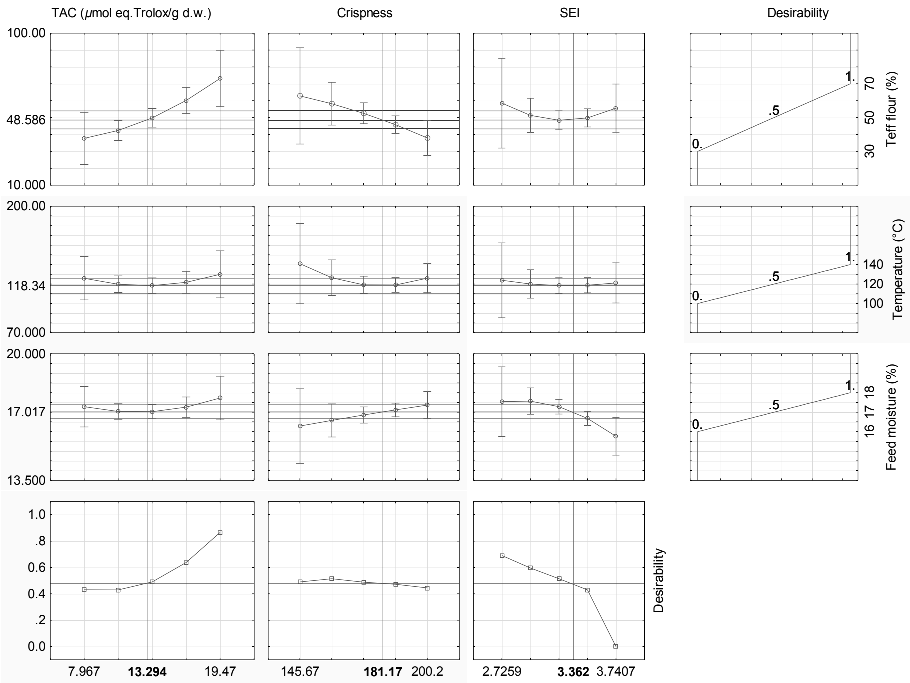


Figure 4.13 Profile of desirability of extruded samples as a function of independent variables



4.3.7 Desirability approach

With the aim to define the best operative conditions in terms of TAC, crispiness and SEI, the changes in desirability of samples as function of the independent variables were analyzed. Crispness and SEI were chosen because they are strictly related to the acceptability of consumers; TAC was selected because of the aim of this study was to develop an healthy breakfast cereal with teff flour and TAC was the only dependent variable that explains the functionality of extruded products. Figure 4.13 shows the desirability profile as a function of each independent variable which were leave free to vary. A maximum desirability value of~ 0.715 was obtained with 70% of teff flour, 16% of feed moisture and a temperature of 120°C. These experimental conditions correspond to a crispness value of 177.66, a SEI value of 3.540 and a TAC of 16.912 µmol eq. Trolox/g d.w.

4.4. Conclusions

The incorporation of teff flour significantly changed the physical and sensory characteristics of extruded cereals. In particular lightness and crispiness were affected by inclusion of teff flour, but process management of extruded cereal breakfast, according to different process conditions, such as feed moisture and temperature of the last barrel, allows creating desirable quality of final products. Increasing the temperature to 120°C, a less hard and crispier products could be obtained, which is more likely to be accepted by consumers. Moreover, varying the process parameters and increasing teff flour addition, microstructural properties of sample change considerably and these characteristics could affect structural properties of extruded products. Also, size distribution functions of both solid phase and voids of extrudates allows a deeper scientific understanding of textural characteristics, some of them linked to sensory preferences. The TAC of extruded samples were strictly related with the teff flour addition. The latter contribute to the obtaining of a healthy and functional product. By using the desirability approach the optimization of the process conditions in terms of crispness, SEI and TAC were obtained.

References

Alam, S. A., Järvinen, J., Kirjoranta, S., Jouppila, K., Poutanen, K., & Sozer, N. (2014). Influence of Particle Size Reduction on Structural and Mechanical Properties of Extruded Rye Bran. Food and Bioprocess Technology, 7(7), 2121-2133. doi: 10.1007/s11947-013-1225-2

Alaunyte, I., Stojceska, V., Plunkett, A., Ainsworth, P., & Derbyshire, E. (2012). Improving the quality of nutrient-rich Teff (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough breadmaking. Journal of Cereal Science, 55(1), 22-30. doi: http://dx.doi.org/10.1016/j.jcs.2011.09.005

Albertson, A. M., Anderson, G. H., Crockett, S. J., & Goebel, M. T. (2003). Ready-to-eat cereal consumption: its relationship with BMI and nutrient intake of children aged 4 to 12 years. Journal of the American Dietetic Association, 103(12), 1613-1619. doi: https://doi.org/10.1016/j.jada.2003.09.020

A.O.A.C. 2000. 'Official Methods on Analysis' 17° ed. Association of Official analytical Chemists, Washington, D.C.

Brennan, M. A., Monro, J. A., & Brennan, C. S. (2008). Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. International Journal of Food Science & Technology, 43(12), 2278-2288. doi: 10.1111/j.1365-2621.2008.01867.x

Collar, C., Jimenez, T., Conte, P., & Fadda, C. (2014). Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads. Carbohydrate Polymers, 113, 149-158. http://dx.doi.org/10.1016/j.carbpol.2014.07.020

Dehghan-Shoar, Z., Hardacre, A. K., & Brennan, C. S. (2010). The physico-chemical characteristics of extruded snacks enriched with tomato lycopene. Food Chemistry, 123(4), 1117-1122. doi: https://doi.org/10.1016/j.foodchem.2010.05.071

Derossi, A., Severini, C., Del Mastro, A., & De Pilli, T. (2015). Study and optimization of osmotic dehydration of cherry tomatoes in complex solution by responce surface methodology and desirability approach. LWT- Food Science and Technology, 60(2), 641-648. https://doi.org/10.1016/j.lwt.2014.10.056

Derringer, G., & Suich, r. (1980). Simultaneous optimization of several response variables. Journal of Quality Technology, 12, 214-219.

Devi, N. L., Shobha, S., Tang, X., Shaur, S. A., Dogan, H., & Alavi, S. (2013). Development of protein-Rich Sorghum-Based Expanded Snacks Using Extrusion Technology. International Journal of Food Properties, 16(2), 263-276. doi: 10.1080/10942912.2011.551865

Di Benedetto, N.A., Alfarano, M., Laus, M.N., Caporizzi, R., Soccio, M., Robbe, C., Flagella, Z., Pastore, D. (2015). Antioxidant Capacity of Durum Wheat Large Flour Particles May Be Evaluated by QUENCHER ABTS Assay by Adopting a Proper Calculation Mode. Cereal Research Communications, 43(4), 682-691.

Ding, Q.-B., Ainsworth, P., Tucker, G., & Marson, H. (2005). The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. Journal of Food Engineering, 66(3), 283-289. doi: http://dx.doi.org/10.1016/j.jfoodeng.2004.03.019

Forsido, S. F., Rupasinghe, H. P. V., & Astatkie, T. (2013). Antioxidant capacity, total phenolics and nutritional content in selected ethiopian staple food ingredients. International Journal of Food Sciences and Nutrition, 64(8), 915-920. doi: 10.3109/09637486.2013.806448

Gebremariam, M. M., Zarnkow, M., & Becker, T. (2014). Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. Journal of Food Science and Technology, 51(11), 2881-2895. doi: 10.1007/s13197-012-0745-5

Guo, W., & Beta, T. (2013). Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. Food Research International, 51(2), 518-525. doi: https://doi.org/10.1016/j.foodres.2013.01.008

Holguín-Acuña, A. L., Carvajal-Millán, E., Santana-Rodríguez, V., Rascón-Chu, A., Márquez-Escalante, J. A., Ponce de León-Renova, N. E., & Gastelum-Franco, G. (2008). Maize bran/oat flour extruded breakfast cereal: A novel source of complex polysaccharides and an antioxidant. Food Chemistry, 111(3), 654-657. doi: https://doi.org/10.1016/j.foodchem.2008.04.034

Ilo, S., Liu, Y., & Berghofer, E. (1999). Extrusion Cooking of Rice Flour and Amaranth Blends. LWT - Food Science and Technology, 32(2), 79-88. doi: http://dx.doi.org/10.1006/fstl.1998.0497

Inglett, G. E., Chen, D., & Liu, S. X. (2016). Physical Properties of Gluten Free Sugar Cookies Containing Teff and Functional Oat Products. Journal of Food Research; Vol 5, No 3 (2016). doi: 10.5539/jfr.v5n3p72

JUNG, K.-j., LEE, H., LEE, S. H., & KIM, J. C. (2017). Retrogradation of heat-gelatinized rice grain in sealed packaging: investigation of moisture relocation. Food Science and Technology, 37, 97-102.

Laus, M.N., Di Benedetto, N.A., Caporizzi, R., Tozzi, D., Soccio, M., Giuzio, L., De Vita, P., Flagella, Z., Pastore, D. (2015). Evaluation of Phenolic antioxidant capacity in grains of modern and old Durum Weath genotypes by the novel QUENCHERABTS approach. Plant Foods for Human Nutrition, 70(2), 207-214

Mäkilä, L., Laaksonen, O., Ramos Diaz, J. M., Vahvaselkä, M., Myllymäki, O., Lehtomäki, I., . . . Kallio, H. (2014). Exploiting blackcurrant juice press residue in extruded snacks. LWT - Food Science and Technology, 57(2), 618-627. doi: https://doi.org/10.1016/j.lwt.2014.02.005

Marti, A., Marengo, M., Bonomi, F., Casiraghi, M. C., Franzetti, L., Pagani, M. A., & Iametti, S. (2017). Molecular features of fermented teff flour relate to its suitability for the production of enriched gluten-free bread. LWT - Food Science and Technology, 78, 296-302. doi: https://doi.org/10.1016/j.lwt.2016.12.042

Mkandawire, N. L., Weier, S. A., Weller, C. L., Jackson, D. S., & Rose, D. J. (2015). Composition, in vitro digestibility, and sensory evaluation of extruded whole grain sorghum breakfast cereals. LWT - Food Science and Technology, 62(1), 662-667. doi: http://dx.doi.org/10.1016/j.lwt.2014.12.051

Moraru, C. I., & Kokini, J. L. (2003). Nucleation and Expansion During Extrusion and Microwave Heating of Cereal Foods. Comprehensive Reviews in Food Science and Food Safety, 2(4), 147-165. doi: 10.1111/j.1541-4337.2003.tb00020.x

Oliveira, L. C., Rosell, C. M., & Steel, C. J. (2015). Effect of the addition of whole-grain wheat flour and of extrusion process parameters on dietary fibre content, starch transformation and mechanical properties of a ready-to-eat breakfast cereal. International Journal of Food Science & Technology, 50(6), 1504-1514. doi: 10.1111/ijfs.12778

Oliveira, L. C., Schmiele, M., & Steel, C. J. (2017). Development of whole grain wheat flour extruded cereal and process impacts on color, expansion, and dry and bowl-life texture. LWT - Food Science and Technology, 75, 261-270. doi: http://dx.doi.org/10.1016/j.lwt.2016.08.064

Potter, R., Stojceska, V., & Plunkett, A. (2013). The use of fruit powders in extruded snacks suitable for Children's diets. LWT - Food Science and Technology, 51(2), 537-544. doi: http://dx.doi.org/10.1016/j.lwt.2012.11.015

Ramos Diaz, J. M., Kirjoranta, S., Tenitz, S., Penttilä, P. A., Serimaa, R., Lampi, A.-M., & Jouppila, K. (2013). Use of amaranth, quinoa and kañiwa in extruded corn-based snacks. Journal of Cereal Science, 58(1), 59-67. doi: https://doi.org/10.1016/j.jcs.2013.04.003

Ramos Diaz, J. M., Suuronen, J.-P., Deegan, K. C., Serimaa, R., Tuorila, H., & Jouppila, K. (2015). Physical and sensory characteristics of corn-based extruded snacks containing amaranth, quinoa and kañiwa flour. LWT - Food Science and Technology, 64(2), 1047-1056. doi: https://doi.org/10.1016/j.lwt.2015.07.011

Robin, F., Dubois, C., Pineau, N., Labat, E., Théoduloz, C., & Curti, D. (2012). Process, structure and texture of extruded whole wheat. Journal of Cereal Science, 56(2), 358-366. doi: https://doi.org/10.1016/j.jcs.2012.02.014

Saeleaw, M., Dürrschmid, K., & Schleining, G. (2012). The effect of extrusion conditions on mechanical-sound and sensory evaluation of rye expanded snack. Journal of Food Engineering, 110(4), 532-540. doi: https://doi.org/10.1016/j.jfoodeng.2012.01.002

Santillán-Moreno, A., Martínez-Bustos, F., Castaño-Tostado, E., & Amaya-Llano, S. L. (2011). Physicochemical Characterization of Extruded Blends of Corn Starch–Whey Protein Concentrate–Agave tequilana Fiber. Food and Bioprocess Technology, 4(5), 797-808. doi: 10.1007/s11947-009-0223-x

Serpen, A., Gokmen, V., Pellegrini, N., Fogliano, V. (2008). Direct measurement of the total antioxidant capacity of cereal products. Journal of Cereal Science, 48, 816-820

Solomon, W. K. (2014). Hydration kinetics of direct expanded tef flour breakfast cereals in water and milk. Food Science & Nutrition, 2(1), 39-45. doi: 10.1002/fsn3.80

Stojceska, V., Ainsworth, P., Plunkett, A., & İbanoğlu, Ş. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. Food Chemistry, 121(1), 156-164. doi: http://dx.doi.org/10.1016/j.foodchem.2009.12.024

Valcarcel, M., Ghatak, R., Bhaduri, S., & Navder, K. P. (2012). Physical, Textural and Sensory Characteristics of Gluten-Free Muffins Prepared with Teff Flour (eragrostis Tef (zucc) Trotter). Journal of the Academy of Nutrition and Dietetics, 112(9), A58. doi: http://dx.doi.org/10.1016/j.jand.2012.06.210

Wójtowicz, A., Mitrus, M., Oniszczuk, T., Mościcki, L., Kręcisz, M., & Oniszczuk, A. (2015). Selected Physical Properties, Texture and Sensory Characteristics of Extruded Breakfast Cereals based on Wholegrain Wheat Flour. Agriculture and Agricultural Science Procedia, 7, 301-308. doi: http://dx.doi.org/10.1016/j.aaspro.2015.12.051

Zhu, F. (2018). Chemical composition and food uses of teff (Eragrostis tef). Food Chemistry, 239(Supplement C), 402-415. doi: https://doi.org/10.1016/j.foodchem.2017.06.101

CHAPTER 5. Exploring the functional and pasting properties of extruded breakfast cereal with teff flour under different extrusion conditions.

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Abstract

There is an increasing concern about functional foods in recent years, especially for gluten-free ready-to-eat products, which generally provide low amount of dietary fiber and phytochemicals. Under this scenario, teff is a fascinating ancient crop, which is finding great approval because of its excellent nutritional profile. We investigated the effects of teff enrichment in rice-based extruded breakfast cereals on their functional and pasting properties. A Box-Behnken design was used to modulate three independent variables such as mass ratio of teff flour (30-70%), feed moisture (16-18%) and end barrel temperature (100-140°C). Insoluble (IDF) and soluble (SDF) dietary fibre, total phenolic compounds (TPC) and total antioxidant capacity (TAC) of free and bound fractions as well as pasting properties, were analyzed. Our results proved that the enrichment of teff flour was capable to improve the functional properties of breakfast cereals, by increasing TPC and TAC in both free and bound fractions, as well as IDF. Extruder end barrel temperature positively affected the free fraction of both TPC and TAC, with estimating regression coefficients of 4.244 and 4.760, respectively, while it had a negative effect on SDF. Feed moisture was inversely related with all parameters except for SDF and IDF, which increased with higher feed moisture content. Considering pasting properties, both feed moisture and barrel temperature decreased final viscosity of extruded samples, with estimated regression of -2.793 and -1.530, respectively.

Our results suggest the potential in use of teff for development of ready-to-eat breakfast cereal by using extrusion-cooking technology with improved functional properties.

Keywords: teff, phenolic compounds, antioxidant capacity, fibre, pasting properties, extrusion

5.1 Introduction

In the last 20 years, consumers' demand for highly nutritious and functional food has increased considerably, because of the consumers' awareness that foods contribute to their health (Forscido et al., 2013; Foschia et al., 2013). People are focusing their attention on foods that naturally contain such functional compounds like fruits, vegetables, pulses and particularly whole grain cereals. Especially, the relation between whole grain cereals and reduction of chronic diseases has received much attention from researchers (Wang et al., 2014). Grain cereals contain a high amount of dietary fibre (DF) consisting in a mixture of complex organic substances, which are classified as soluble (oligosaccharides, pectin, β-glucans, etc.) and insoluble (cellulose, hemicelluloses and lignin) fractions (Foschia et al., 2013). Both soluble and insoluble fractions are capable to provide many physiological benefits such as weight control, constipation, cholesterol and fat binding, decrease in blood glucose level, as well as to the prevention of cardiovascular diseases and certain cancers (Potter et al., 2013; Foschia et al., 2013). However, apart from the content in fibre, whole grain cereals are a good source of phenols, which are mainly located in the pericarp of the kernel (Shaidi et al., 2013). They play an important role in protecting humans from many chronic diseases, and they may even have anti-aging properties (Tufan et al., 2013; Obrenovich et al., 2010). This nutritional profile of whole grains make it an ideal ingredient to improve the functional quality of extruded products.

However, a public concern is the great increase of pathologies associated with gluten intake, such as food allergy, celiac disease and gluten sensitivity (Rossel et al., 2014). Till now, in people who are suffering from these pathologies, the only effective therapy is based on a gluten-free diet, which is often characterized by an unbalanced intake of different micronutrients (Rossel et al., 2014). Gluten-free products are commonly obtained from formulations of refined cereals, rice, maize and/or starch, providing a low amount of fibre and phytochemicals (Stojecka et al., 2010).

Under this scenario the use of teff (*Eragrostis tef*) could be a useful option to improve the nutritional and functional pattern of gluten-free products. Teff is an ancient minor cereal crop, used in Ethiopia as a staple food (Baye et al., 2014), which is now recognized in the modern age. It has an exceptional nutritional profile because of its significant content of minerals, vitamins and for its excellent amino acids composition (Zhu 2018; Inglett et al., 2016;). Moreover, due to the fact that is the smallest cereal in the world, teff cannot be decorticated, so it is consumed as wholegrain, providing an amount of fiber content that is higher than in other gluten containing and gluten-free cereals. Furthermore, it is generally assumed as a good source of phenols (Dykes and Rooney, 2007; Gebremariam et al., 2014). In Ethiopia, where it is daily consumed, it contributes to the increase of fibre and phenol intake in the human diet (Baye et al., 2014).

As lifestyle continues to become more hectic, an increased number of people are turning to ready-to-eat (RTE) snack foods. Extrusion-cooking has been investigated as a mean of producing snacks, meeting the dietary requirements of particular groups of population (Potter et al., 2013). This technology seems promising in creating new gluten-free products with higher nutritional values. Ibanou et al. (2006) investigated the use of extrusion-cooking to produce snacks, meeting the requirements for a gluten-free diet. Stojeska et al. (2010) increased the level of total dietary fibre in extruded gluten-free products by incorporating a number of different fruits and vegetables. In gluten-free extruded products corn or pseudo-cereals has been used before (Ramos-Diaz et al., 2015; Holguin-Acuna et al., 2008), but only few studies investigated the use of teff flour in extruded products (Solomon et al., 2013; Stojeska et al., 2010).

The aim of this study was to investigate the use of teff flour in RTE cereal breakfast as a novel source of functional compounds. In particular the effect of some extrusion process variables on the IDF and SDF content, antioxidant capacity, phenolic content and pasting properties of the extruded samples were studied.

5.2 Materials and methods

5.2.1 Materials and reagents

5.2.1.1 Raw materials

Rice and teff flours were supplied by Molino Maraldi sas (Cesena, Italy) and stored at 4°C until use. Three different blends of these flours were prepared by using mass ratios between teff and rice flours of 30:70, 50:50, 70:30.

5.2.1.2 Chemical reagents

Sulphuric acid, petroleum ether, celite, hydrochloric Acid fumant 37%, sodium hydroxide, tris-hydrochloriid pufferan, MES-pufferan, Iron (II) sulphate heptaidrate, potassium persulfate, acetic acid ethylester, sodium sulfate, Folin-Ciocalteu’s phenol reagent, sodium carbonate, sodium acetate trihydrate, acetic acid 100% were purchased from Carl Roth GmbH Co. KG (Karlsruhe, Germany). Hydrochloric Acid 0.1 mol, 2,2′-Azino-bis(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, methanol, Iron (III) chloride hexahydrate pure, 2,4,6-Tris(2-pyridyl)-s-triazine were obtained from Sigma Aldrich (Germany). Ethanol 96% was purchased from Prima (Spillern, Austria), while acetone from Acros Organics (Geel, Belgium). From Fluka Chemie (Switzerland) was obtained 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX).

5.2.2 Sample preparation and extrusion experiment

The sample preparation and the extrusion experiment were previously described in chapter 4). Briefly RTE (ready-to-eat) breakfast cereals were obtained by using a co-rotating twin-screw extruder BC-21 CLEXTRAL (Firminy, France). A Box-Behnken design was applied to study the effect of each independent variable (end barrel temperature (°C) from 100 to 140 °C, feed moisture of the blend (%) from 16 to 18% and teff flour (%) from 30 to 70%) on some functional and pasting properties of extruded samples. The coded and the real values of the independent variables are shown in Table 5.1. After the extrusion process the samples were dried at 50°C in a forced-air convention oven to ~ 6% of moisture content for storage. Then they were packed into polyethylene bags and stored in refrigerated conditions (4°C) until use.

5.2.3 Chemical analysis

5.2.3.1 Extraction of bioactive compounds

Extraction of free and bound phenols were developed with the method of Abguri et al. (2015) with some minor modifications. 0.5 g of each samples were added with methanol and each tube containing samples were closed under N_2 to avoid oxidation of bioactive compounds. Then all samples were vortexed for 20 seconds, incubated for 5 minutes at 30°C in an ultrasound bath Baudeline Sonorex RK100H (Germany), then they were overshaked for additional 5 minutes. At the end, the samples were centrifuged for 10 minutes at 4000 rpm and 4°C using a centrifuge mod. 5810R (Hamburg, Germany). The supernatant was collected as free extract and stored at -18°C until use. The solid residues were used to extract bound phenolic compounds. The residues were subjected to basic hydrolysis by adding 3 ml of distilled water, 5 ml of NaOH 5M and 5 mL of NaOH 10 M and overshaked overnight in the dark with a MultiRotator Grant-Bio PTR-60 (Cambs, England). After this step the pH was adjusted to~2 with HCl 8 M and it was added with 15 mL of ethylacetate and then centrifuged at 4000 rpm for 10 minutes 3 times in order to recover the supernatant. The latter was filtered with Na_2SO_4 and then evaporated to drynesss under vacuum at 40°C. The fraction of phenolic compounds so obtained were reconstituted in methanol 50% (v/v) and stored under nitrogen atmosphere at -18°C until used for further analysis. During all stages, extract were protected from light by covering the containers with aluminum foil. Free and bound extracts were used for the determination of phenolic content and for the analysis of the antioxidant capacity with TEAC and FRAP methods, as described below.

5.2.3.2 Total Antioxidant Capacity (TAC) with TEAC method

The TEAC assay was applied as described in Re et al. (1998) and modified as reported in Pastore et al. (2009) and Laus et al. (2012). The $ABTS^{\cdot+}$ radical cation was generated by chemical oxidation with potassium persulfate and then diluted with methanol:water (50:50, v/v) in order to obtain an absorbance value at 734 nm of about 0.70±0.02. The absorbance was detected with the spectrophotometer Hitachi mod. U-1100 (Tokyo, Japan). Trolox was used as a standard to obtain a proper calibration curve. Measurements of both free and bound fractions of raw blends and extruded samples were carried out in triplicates.

5.2.3.3 Total Antioxidant Capacity (TAC) with FRAP method

The ferric reducing antioxidant power was determined according to the method of Benzie and Strain (1996) with some modifications. 0.2 mL of each extract was mixed with 1.3 mL of FRAP working reagent, consisting of acetate buffer 0.3 M (pH 3.6), TPTZ solution 10 mM and ferric chloride 20 mM in the ratio 10:1:1, respectively. Then the samples were stored for 30 minutes at 37°C and the absorbance were read at 595 nm. The TAC was calculated based on a methanol solution of $FeSO_4$ x $7H_2O$, as a standard curve. Each sample, raw materials and extruded products, were run in triplicate for both free and bound fractions.

5.2.3.4 Total Phenolic Content (TPC)

The total phenolic content of each phenolic fraction was determined using the colorimetric Folin-Ciocalteau method described by Singleton and Rossi (1965) with slight modification as reported by Laus et al. (2012). It measures the absorbance of the blue coloured complex formed as a result of the reaction between phenolic compounds and the Folin-Ciocalteau reagent. The absorbance of these blue coloured complex, which can be measured spectrophotometrically, is proportional to the reacting phenolic hydroxyl groups (Guo and Beta, 2013).

The phenolic compounds were determined by means of a proper calibration curve using gallic acid as standard. The assay was run in triplicates for raw blend and extruded samples.

5.2.3.5 Insoluble (IDF) and Soluble (SDF) Dietary Fibre

The SDF and IDF were determined according to the AACC method 32-07.01 (AACC, 2012). Briefly, duplicates of samples were cooked at ~100°C with heat stable α- amylase to give gelatinization, hydrolysis and depolymerisation of starch; incubated at 60°C with protease in order to solubilise and depolymerise proteins, and amyloglucosidase to hydrolise starch fragments to glucose. Meagazyme Total Dietary Fibre Assay kit (K-TDRF-200A, Meagazyme, Ireland) was used. IDF was filtered and then the residue was washed with 10 mL of warm distilled water and 10 mL of ethanol 95%, twice. Combined solution of filtrate and water-ethanol washing was then precipitated with 4 volumes of 95% ethanol warmed at 60°C for the determination of SDF. The SDF precipitate is then filtered, washed twice with 15 mL of 78% ethanol and 95% ethanol, and dried. One duplicate was analyzed for protein and the other is incubated at 525°C to determine ash. Both SDF and IDF residues were corrected for protein, ash and blank for the final calculation of IDF and SDF. Three replicates were assessed for each raw materials and extruded samples; results were expressed in percentage on dry basis.

5.2.4 Pasting properties

Pasting properties of raw materials and extruded samples were evaluated by using a Rapid Visco Analysed 4500 (RVA 4500, Perten Instrument, CITY, Sweden). An amount of 3.5 g of each sample, previously corrected for its own moisture, was dispersed into 25 mL of distilled water. The viscosity profile was then monitored during the heating and cooling stages and recorded with the software Thermocline for Windows, version 3 (TCV, Perten Instrument, CITY, Sweden). Set-up *Extrusion 1* no-alcohol and *Standard 1* profile, reported in Table 5.2, were used to analyze the pasting properties of extruded samples and raw materials, respectively. All the samples were analyzed in triplicates. Pasting parameter evaluated included peak viscosity (cP)- maximum viscosity after the heating portion of the test, hold peak (cP)- minimum viscosity after the cooling start, final peak (cP)- viscosity at the end of the test.

5.2.5 Statistical analysis

The effects of each independent variable on quality indices were determined by ANOVA and Fisher's least significance test with a significance of 0.05. Moreover, experimental data were fitted by the polynomial model of Eq.1 with the aim to evaluate linear, non-linear and interactive effect of independent variables.

$y = B_0 + \sum B_i \times_i + \sum B_{ii} \, x^2{}_{ii} + \sum B_{ij} \times_{ij}$ (Eq.1)

Where, y is the response variable; B_0, B_i, B_{ii}, B_{ij}, are the regression coefficients for constant, linear, quadratic and interaction regression; x_i, are the coded values for linear effects of the independent variables; x^2 are the coded values for quadratic effects of the independent variables; $\times_{ij}$ are the coded values for interaction effects of the independent variables.

In addition a correlation analysis among all the data was performed. For all the analysis Statistica software ver. 10 (Statsoft, Tulsa, USA) was used.

Table 5.2. Description of the set-up *Extrusion 1* no-alcohol and *Standard 1* profiles used in the RVA analysis.

Stage	***Standard 1* profile**	***Extrusion 1* no-alcohol profile**
Initial temperature, °C	50	25
Initial holding time, min	2	2
Heating time, min	6	5
Maximum temperature, °C	95	95
Hold at maximum temperature, min	4	3
Cooling time, min	4	5
Final temperature, °C	50	25
Final holding time, min	4	5
Total test time, min	20	20

Table 5.1. Coded and real values of the Box-Behnken design and fibre content of raw and extruded samples.

SAMPLE	CODED VALUES			REAL VALUES			INSOLUBLE FIBRE (%)	SOLUBLE FIBRE (%)	TOTAL DIETARY FIBRE (%)
	X_1	X_2	X_3	Teff flour (%)	T (°C)	Feed moisture (%)			
1	1	-1	0	70	100	17	4.614±0.295	0.746±0.201	5.359±0.496
2	-1	-1	0	30	100	17	2.008±0.075	0.468±0.008	2.476±0.083
3	1	1	0	70	140	17	4.187±0.163	0.381±0.073	4.569±0.236
4	-1	1	0	30	140	17	2.524±0.117	0.288±0.056	2.811±0.173
5	1	0	-1	70	120	16	4.012±0.305	0.386±0.066	4.398±0.371
6	-1	0	-1	30	120	16	2.627±0.029	0.326±0.066	2.953±0.095
7	1	0	1	70	120	18	4.157±0.032	0.531±0.032	4.689±0.063
8	-1	0	1	30	120	18	2.670±0.157	0.380±0.000	3.050±0.157
9	0	-1	-1	50	100	16	3.166±0.108	0.325±0.016	3.491±0.124
10	0	1	-1	50	140	16	2.574±0.089	0.310±0.017	2.884±0.105
11	0	-1	1	50	100	18	3.677±0.246	0.463±0.050	4.140±0.297
12	0	1	1	50	140	18	3.366±0.055	0.286±0.017	3.652±0.072
13	0	0	0	50	120	17	3.045±0.029	0.484±0.049	3.530±0.077
14	0	0	0	50	120	17	2.945±0.175	0.477±0.013	3.421±0.188
15	0	0	0	50	120	17	3.071±0.205	0.475±0.060	3.546±0.265
RAW MATERIALS	70 % teff flour						4.740±0.012	0.664±0.061	5.404±0.045
	50 % teff flour						3.535±0.095	0.493±0.036	4.028±0.066
	30 % teff flor						2.331±0.099	0.321±0.014	0.922±0.038

Table 5.3. Phenolic content and antioxidant capacity of raw and extruded samples.

SAMPLE		Total Phenolic Compounds (mg gallic acid eq./g d.w.)		Total Antioxidant Capacity- ABTS (umoli Trolox Eq./ g d.w.)		Total Antioxidant Capacity- FRAP (µmol Fe2+Eq /g d.w.)	
		BOUND	FREE	BOUND	FREE	BOUND	
1		0.186±0.004	0.687±0.027	3.653±0.279	45.018±4.324	0.714±0.026	5.094±0.102
2		0.150±0.015	0.259±0.005	1.828±0.132	32.526±1.340	0.387±0.019	1.903±0.068
3		0.233±0.009	0.647±0.016	2.002±0.131	43.925±1.978	1.053±0.046	5.109±0.104
4		0.153±0.006	0.200±0.006	2.492±0.258	37.500±1.755	0.510±0.034	2.924±0.070
5		0.209±0.001	0.390±0.026	3.951±0.372	41.057±1.633	0.933±0.030	5.092±0.137
6		0.165±0.010	0.155±0.018	2.972±0.264	14.467±1.949	0.576±0.008	0.775±0.016
7		0.189±0.011	0.695±0.019	3.431±0.348	47.746±4.106	0.742±0.025	4.795±0.036
8		0.146±0.003	0.196±0.017	2.139±0.254	33.233±2.105	0.489±0.070	1.310±0.058
9		0.180±0.012	0.678±0.013	3.757±0.315	50.341±1.288	0.832±0.034	5.048±0.061
10		0.210±0.027	0.465±0.019	3.784±0.373	33.622±1.629	0.881±0.013	3.434±0.056
11		0.167±0.015	0.125±0.011	2.924±0.259	10.820±1.304	0.525±0.038	0.429±0.006
12		0.176±0.014	0.127±0.005	2.630±0.256	11.588±1.264	0.572±0.006	0.539±0.008
13		0.179±0.009	0.621±0.022	3.106±0.304	39.287±1.269	0.614±0.007	4.350±0.007
14		0.173±0.001	0.598±0.022	2.811±0.262	37.170±2.019	0.551±0.010	3.948±0.044
15		0.184±0.004	0.537±0.019	2.569±0.243	37.531±3.609	0.569±0.022	3.977±0.062
RAW MATERIALS	70 % teff flour	1.131±0.066	0.578±0.008	22.757±0.825	41.684±3.695	7.695±0.185	4.157±0.063
	50 % teff flour	0.889±0.048	0.455±0.021	17.506±0.451	32.966±2.582	5.897±0.096	3.120±0.047
	30 % teff flour	0.648±0.022	0.332±0.007	12.255±0.623	24.247±2.058	4.100±0.028	2.082±0.021

5.3 Results and discussion

5.3.1 Total Phenolic Content (TPC)

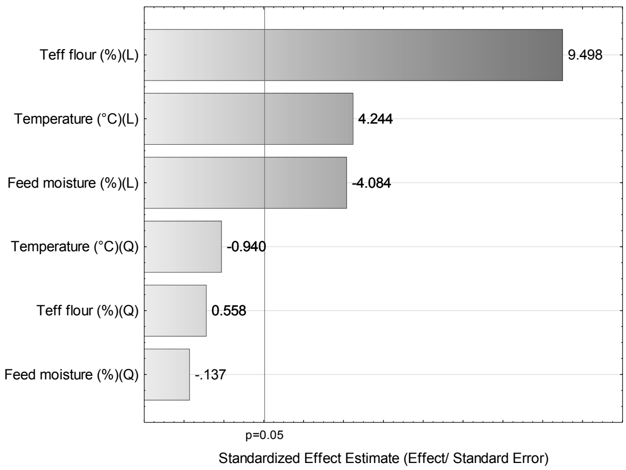
In the present study, free phenols of extruded samples ranged from 0.146±0.003 to 0.233±0.009 mg gallic acid eq./g d.w. (Table 5.3). The free phenolic content decreased after extrusion-cooking in all the samples, with significant differences between samples with various teff content. In particular the highest loss of about 82% was detected in extruded samples with 70% of teff flour, while a loss of about 76% was observed in samples with 30 % of teff flour. However, in both cases, free phenolic content was highly affected by the extrusion-cooking process. Several studies reported significant losses of phenolic compounds after extrusion-cooking (Wang et al., 2013; Viscidi et al., 2004; Dlamini et al., 2007; Mora-Rochin et al., 2010). These reductions may be attributed either to the decomposition of phenolic compounds under the high extrusion temperature, or the alteration in molecular structure of phenolic compounds that may lead to a reduction in their chemical reactivity (Sharma et al., 2012; Wang et al., 2013). It is well known that temperatures over 80°C may destroy or alter the natural properties of phenolic compounds, which are less resistant to heat. In this study temperatures from 100 to 140°C were reached, as described in section 5.2.2, so probably degradation and/or oxidation of free phenolic compounds occurred with the extrusion process.

On the contrary bound phenols increased after extrusion-cooking (Table 5.3). Several authors reported similar results on bound phenols of extruded products (Zilick et al., 2014; Gumul et al., 2006). Some studies stated that extrusion-cooking is effective in retaining higher levels of phytochemicals, in particular ferulic acid (Wang et al., 2013). Ferulic acid is the most abundant phenolic acid, which is bound to the arabinoxylans. As it was reported by Vitaglione et al. (2008) and Gokmen et al. (2009), about 95% of grain phenolic compounds are covalently bound to cell wall polysaccharides through ester bound, so they represent the highest portion of phenols in cereals. In fact, also in this study, bound phenolic content, ranging from 0.125±0.011 to 0.695±0.019 mg gallic acid eq./g d.w., were higher than free phenols (Table 5.3).

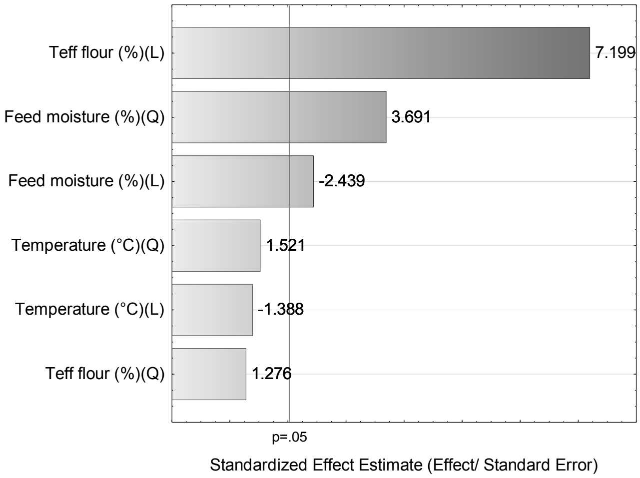
By analyzing the effect of the independent variables on both free and bound TPC, teff flour had a positive and linear effect of 9.49 and 7.19, respectively (Figure 5.1), which means that an increasing amount of teff flour led to an increment of TPC content of the extruded samples. These results were expected because of the high content of the raw material with 70% of teff flour respective to the blend with 30% of teff flour. In fact, it is reported that teff grain contains more polyphenols than white rice (Hager et al., 2012; Dykes and Rooney, 2007). Moreover feed moisture had a linear and negative effect on TPC of both free and bound fractions, with estimated regression values of -4.08 and -2.44, respectively. The higher moisture content probably promoted phenolic and tannin polymerization leading to a low value of these compounds (Remy et al., 2000). Finally, temperature produced a linear and positive effect of 4.24 on TPC free fraction (Figure 5.1a). The same trend of TPC was observed by Zilick et al. (2014) by increasing extrusion-cooking temperature from 100 to 140°C, which was the same temperature as reached in our study. However literature data on the effect of extrusion-cooking variables on TPC content in teff-based products are scarcely available.

Figure 5.1 Estimated Effects of independent variables on Total Phenolic Content of FREE (a) and BOUND (b) fractions of extruded samples.

a)



b)



5.3.2 Total Antioxidant Capacity (TAC)

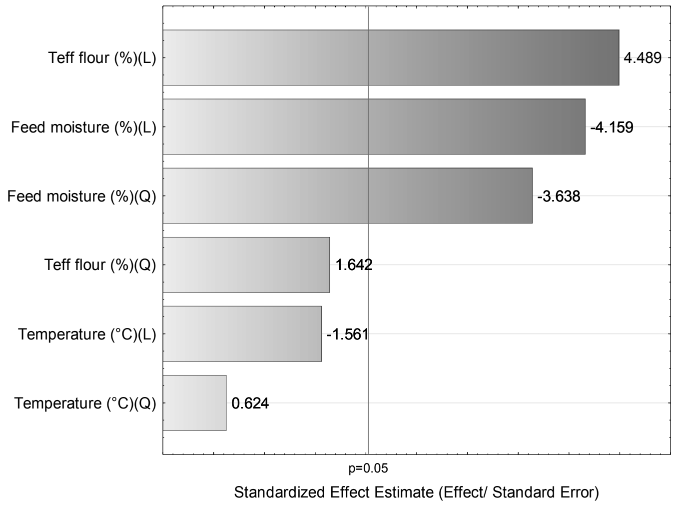
As it is well reported, several factors could affect the antioxidant capacity of food materials, such as solvent extraction, method used, except of the influence of the raw material. For these reasons it seemed necessary to perform different TAC procedures in order to take into account various antioxidant actions (Kotaskova et al., 2016; Deng et al., 2012). TEAC method, which measures the ABTS scavenging ability, and FRAP assay, which provides the capacity of antioxidants to reduce ferric ions, were performed (Forscido et al., 2013; Kotaskova et al., 2016).

As reported for TPC, the Total Antioxidant Capacity (TAC) measured with both ABTS and FRAP assay, was higher in bound fraction than in free fraction of extruded samples (Table 5.3). Furthermore in the free fraction a decrease of the antioxidant activity was detected, while in the bound fraction an increase of TAC occurred after the extrusion-cooking process. These results were in accordance with the TPC contents previously discussed. Some studies reported extrusion-cooking could significantly reduce antioxidant activity (Wang et al., 2013; Sharma et al., 2012; Anton et al., 2009; Ozer et al., 2006). In addition, the decrease of free phenolic content may be the reason for the decrease of the TAC of the free fraction after extrusion-cooking. An interesting result was that the reduction of TAC in the free fractions was higher in samples with 70% of teff flour compared to samples with 30% of teff flours, in particular when measured by the TEAC method. As suggested from the study of Dehghan-Shoar et al. (2010), probably high starch content may provide some protection for bioactive compounds. In our case teff flour is a cereal grain with lower starch content and higher protein content than rice flour (Hager et al., 2012), which probably explains the different retention of antioxidant activity. On the other hand, an explanation for the increase of TAC in the bound fraction of extruded sample was that new antioxidants may be developed as a result of the Maillard reaction occurring during extrusion-cooking or that inactive antioxidants were transformed into active antioxidants due to non enzymatic browning (Pokorny et al., 2006; Stojceska et al., 2009). The non-enzymatic browning, well known as Maillard reaction, refers to a complex series of reactions, which starts with the reaction between amines and carbonyl compounds at elevated temperatures (Friedman, 1996).

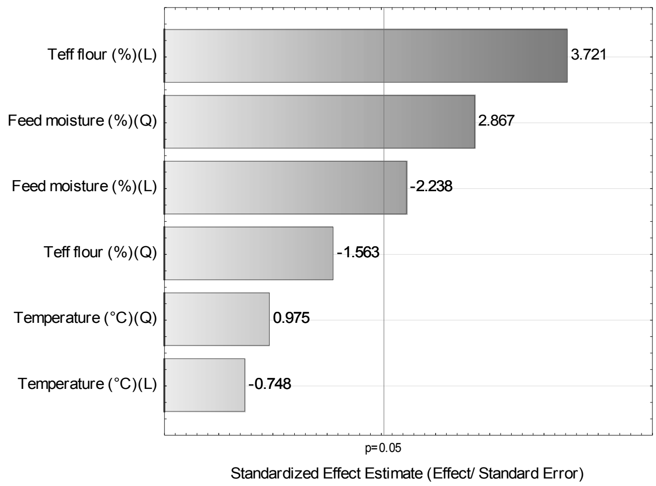
In both methods used to measure the total antioxidant capacity and in both analysed fractions, teff flour amount had a linear and positive effect (Figure 5.2). The highest effect was observed within the FRAP method with values of 12.61 and 8.92 for free and bound fractions, respectively (Figure 5.2c, 5.2d). Feed moisture had a linear and negative effect in both methods used and fractions analysed. As reported for the TPC, the higher feed moisture may lead to a polymerization of phenolic and tannin compounds, which affected extractability of phenols and in turn reduced antioxidant capacity. The effect of temperature was significant ($p<0.05$) only in the TAC of free fraction analysed with FRAP method. All the effects found in TAC analyses were similar to that found in the TPC assay. In fact, correlation analysis demonstrated that TPC of free factions were correlated with TEAC values ($R^2=0.315$, $p<0.05$) and with FRAP values ($R^2=0.856$, $p<0.001$). In addition, TPC of bound fractions were correlated with both antioxidant methods with values of 0.384 ($p<0.01$) and 0.908 ($p<0.001$) for TEAC and FRAP assay, respectively. These correlations were reported in many studies (Wang et al., 2013; Kotaskova et al., 2016), suggesting that phenolic compounds could be the major contributors to antioxidant capacity. It should be stated that a stronger correlation between TPC and FRAP values was found than between TPC and TEAC values. The reducing power of antioxidant compounds was associated with the presence of reductones. The phenolic compounds acted in a similar way as the reductones by donating electrons and by reacting with free radicals. The latter reaction lead to a termination of the free radical chain reaction, and converting the free radical into more stable products (Omwamba et al., 2010). This was probably the reason of such strong correlation between phenolic compounds and FRAP values.

Figure 5.2 Estimated Effects of independent variables on Total Antioxidant Activity (TAC) with TEAC method of FREE (a) and BOUND (b) fractions and FRAP method of FREE (c) and BOUND (d) fractions of extruded samples.

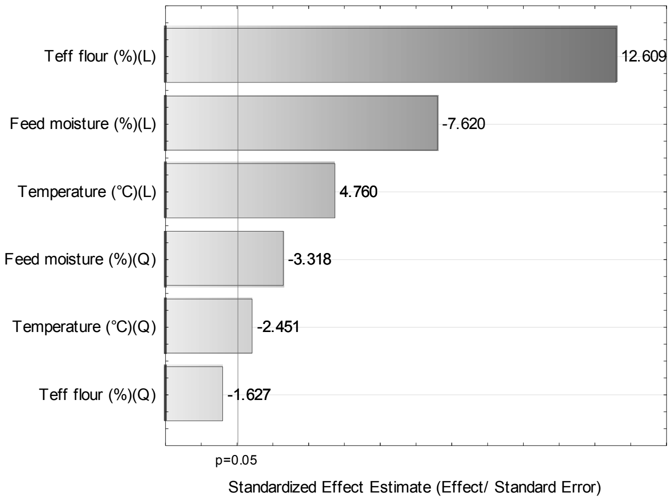
a)



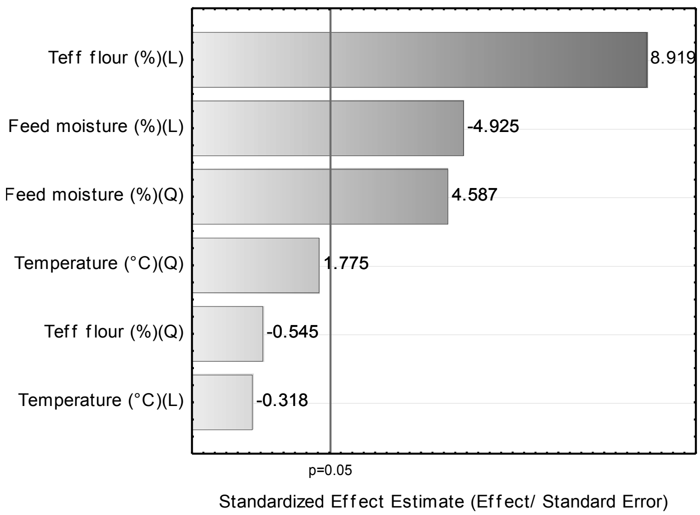
b)



c)



d)



5.3.3 Soluble (SDF) and Insoluble (IDF) Dietary Fibre

It has to be pointed out that a general decrease of both fractions of the dietary fibre content, IDF and SDF, occurred after extrusion-cooking. Several studies reported a decrease of dietary fibre after the extrusion process (Oliveira et al., 2015; Zhang et al., 2011; Stojeska et al., 2009). This was probably a result of solubilisation and the degrading of pectic substances, as a consequence of the break of the glucosidic linkages in the dietary fibre polysaccharides during the process. IDF values of extruded samples, which ranged from

2.008±0.075 % to 4.614±0.295 %, were higher than SDF values, with lay between 0.286±0.017 and 0.746±0.201 (Table 5.1). These results reflect the higher content of insoluble fibre in raw material compared to the soluble fraction (Table 5.1).

The fibre-enriching effect of teff flour was confirmed. As expected, teff flour had a positive and linear effect of 15.395 and 4.048 on insoluble and soluble dietary fibre, respectively (Figure 5.3); similar differences after addition of high-fibre flours were reported by Oliveira et al. (2015). Feed moisture had a positive and linear effect on IDF with a value of 3.25, while had a positive and non linear effect on SDF of 3.73 (Figure 5.3). Stojceska et al., (2009) also highlighted an increasing amount of TDF by incrementing the moisture content of extruded samples of up to 15%. In our study a moisture content of 16-18% was used. The authors attributed the increase in fibre at increased feed moisture to the formation of resistant starch. A resistant starch is a portion of starch and starch products resistant to amylolytic enzymes (Eerlingen & Delcour, 1995) and is therefore indigestible. It is now regarded as a component of dietary fibre (AACC International, 2010). Resistant starch formation is induced as a result of gelatinisation during cooking and retrogradation on cooling (Kim et al., 2006). During the cooking steps of starch products, water penetrates the starch granules and separates the amylose and amylopectin chains from each other leading to the swelling and the softening of the granule, which is known as gelatinisation. On cooling, the process known as 'retrogradation' occurs, which is a result of the reorganisation of the amylose and amylopectin chains (McGee, 2004). Probably the higher feed moisture promoted a higher penetration of water in the starch granules leading to a complete gelatinization and thus to a higher retrogradation, thus higher amount of resistant starch. Also some other studies observed an increase of SDF and IDF with higher feed moisture content (Jing et al., 2013; Kim & Ryu, 2013) in their extruded products.

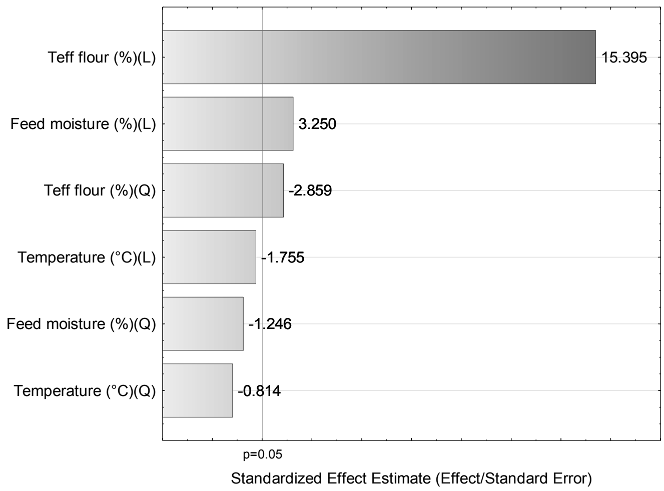
Temperature had a linear and negative effect on SDF with values of -5.120 (Figure 5.3), while no significant effects of this process parameter were observed for IDF. The high end-barrel temperatures reached in this study were probably responsible for the degradation of some soluble polysaccharides.

IDF was highly correlated with FRAP values of free (R^2=0.571, $p<0.001$) and bound fractions (R^2=0.485, $p<0.001$), while SDF was only correlated to FRAP values of bound extracts (R^2=0.325, $p<0.05$). This was probably due to the presence of antioxidant compounds associated with polysaccharides, which are responsible of some healthy effects attributed to dietary fibre (Foschia et al., 2013).

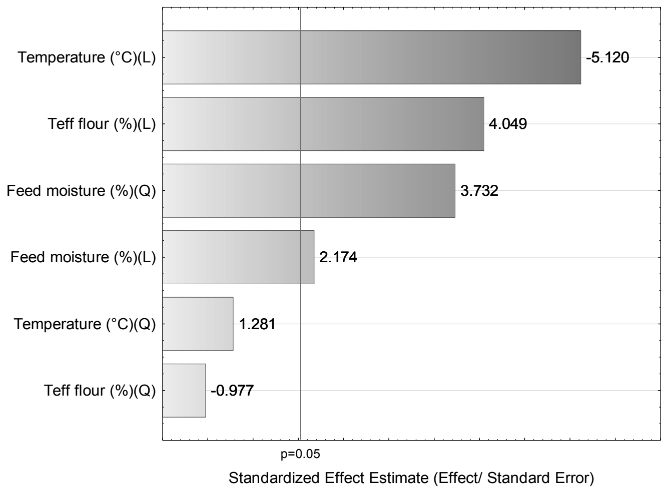
According to current recommendations, the amount of TDF daily intake should not be less than 25g/d (SINU, 2014). To claim that a food is a 'source of dietary fibre' or 'high in fibre', it should contain at least 3 or 6 g of fibre per 100 g in the end product, respectively (European Commission, 2006). The majority of our extruded samples could achieve the claim 'source of dietary fibre' (Table 5.1).

Figure 5.3 Estimated Effects of independent variables on: a) Insoluble Dietary Fibre- IDF (%) and b) Soluble Dietary Fibre- SDF (%) of extruded samples.

a)



b)



5.3.4 Pasting properties

Evaluation of pasting properties is of fundamental importance to provide useful information for food processing. In particular, with the RVA data, knowledge on the impact of extrusion variables and teff flour addition on starch technological properties were obtained, because the RVA test measured the after-effects of gelatinization. The pasting character is basically determined by the starch granule composition and structure as well as by the non-starch flour components (Bultosa 2007). In extruded products the RVA analysis reflects the nature and the amount of thermal and mechanical conversion of these products (Whalen, 2009).

The first parameter investigated was peak viscosity, which was recorded at about 6 minutes for native flour and at about 3 minutes for extruded samples. It represents the swelling of the starch granules as a consequence of the movement of water to the interior of the starch granules. The granules absorb and bind more water as they swell, reducing the available water and causing physical interactions between granules. These interactions, known as the phenomenon of 'pasting', caused the sudden increase in viscosity during the heating phase of the test. The peak viscosity of the raw materials was 3101, 2694 and 2286 cP for blends with 30, 50 and 70% of teff flour, respectively (data not shown). These results were expected, because of the reduction of the starch content available to swell in high teff flour blends (Symons, 2004). The peak viscosity of the extruded products, ranging from 511 to 829 Cp, was lower than the native flour). These values reveal that during the extrusion process starch gelatinisation occurs, observed mainly by the lower peak time and peak viscosity of the extruded products.

As the test mixture continue to be heated, granules of starch begin to swell more significantly and start to break down with an ensuing reduction in the viscosity, because of the melting of the crystalline regions, which lead to the collapse and the total disruption of the starch granule. At this stage the hold peak was recorded. Hold peak of extruded samples were significantly lower than of the raw materials, showing that the extruded products were completely gelatinised. These data were in agreement with results found in literature (Oliveira et al., 2015). Finally the viscosity decrease ceases after the cooling stage begins. The phenomenon that occurs at this stage is often referred to as 'retrogradation'. As the temperature falls, glucan chains from the starch become entangled with each other and form a gel, which leads to an increase of the viscosity up to a plateau value. So the final peak represents the degree of retrogradation.

Regarding the effect of the independent variables on the extruded samples it was possible to observe a general linear and negative effect of teff flour amount and moisture content on all the parameters investigated (Table 5.4). First, it has to be considered that raw materials exhibited different viscosity; the same trend in the reduction of viscosity with increasing content of teff flour was therefore observed in extruded samples. Also, Ilo et al. (1999) founded that, by increasing the ratio of amaranth flour to rice flour, the peak viscosity decreased. Moreover it is well known that peak viscosity increases as moisture content decreases (Whalen, 2009), because more water results in higher gelatinisation. In contrast, temperature had a positive and linear effect of 5.803 on peak viscosity and a negative and linear effect of -1.530 on final peak (Table 5.4). Also, Oliveira et al (2015) highlighted similar results of the effect of temperature on their extruded breakfast cereals made from corn and whole wheat.

Based on the obtained results, by increasing the teff flour content, feed moisture and temperature, lower values of the final viscosity were obtained. So, under these extrusion parameter conditions a completely gelatinised products was achieved. In addition, final viscosity was highly and negatively related to IDF values (R^2=-0.381, $p<0.01$), indicating that samples with high fibre content exhibited a lower final viscosity. This was in agreement with the beforehand discussions, where it was stated that low final viscosity values represent high degrees of retrogradation. In fact, final viscosity and IDF were affected by all the independent variables (Figure 5.2, Table 5.4), the inverse relationship between IDF and final viscosity was thus explained. Furthermore, retrograded starch could be detected as dietary fibre.

Table 5.4 Estimated Effects of the independent variables on pasting properties of extruded samples

Independent variables	Estimated effects		
	Peak viscosity (cP)	**Hold peak (cP)**	**Final peak (cP)**
Teff flour (%) (L)	-3.147*	-10.770*	-3.133*
Teff flour (%) (Q)	1.369	1.569	0.269
Feed moisture (%) (L)	-0.748	-3.687*	-2.793*
Feed moisture (%) (Q)	2.551*	-4.800*	-4.714*
Temperature (°C) (L)	5.803*	-0.040	-1.530
Temperature (°C) (Q)	-0.299	1.788	2.283*
R^2	0.639	0.809	0.565

*Statistically significant at $p<0.05$

5.4 Conclusion

The presented results suggest that teff flour served as a good source of polyphenols, which are the major contributors to the antioxidant capacity, in extruded ready-to-eat cereal breakfast. The formation of gluten-free expanded products with high antioxidant capacity could be achieved by controlling extrusion-cooking conditions, such as temperature and feed moisture. Under optimal conditions, the extrusion-cooking technology is a suitable method for the preparation of gluten-free products with high level of dietary fibre. The final dietary fibre content was mainly dependent on the raw materials, but it could be controlled by changing the extrusion conditions, such as moisture content and temperature.

Also pasting properties were highly affected by all the independent variables investigated in this study. In particular final viscosity was inversely influenced by an increasing amount of teff flour, feed moisture and extruder end-barrel temperature.

Due to their functional properties, teff-based extruded samples could be useful for increasing dietary fibre intake and to provide food products with high phenolic compounds and antioxidant capacity.

References

Abugri DA, Akudago JA, Pritchett G, Russell AE, McElhenney WH (2015) Comparison of Phytochemical Compositions of *Sorghum Bicolor* (L) Moench Red Flour and Pale Brown Leaves. J Food Sci Nutr 1: 003.

Adedokun, M. O., & Itiola, O. A. (2010). Material properties and compaction characteristics of natural and pregelatinized forms of four starches. Carbohydrate Polymers, 79(4), 818-824. doi: https://doi.org/10.1016/j.carbpol.2009.10.009

American Association of Cereal Chemists (2012). Approved methods of the American Association of Cereal hemists. St. Paul: American Association of Cereal Chemists.

American Association of Cereal Chemists, 2010. Approved method of AACC. Methods 38-12A, 44-15A, 54-21, 26-41, eleventh ed AACCI, the Association, S. Paul (MN)

Anton, A. A., Gary Fulcher, R., & Arntfield, S. D. (2009). Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (Phaseolus vulgaris L.) flour: Effects of bean addition and extrusion cooking. Food Chemistry, 113(4), 989-996. doi: https://doi.org/10.1016/j.foodchem.2008.08.050

Baye K. (2014) Teff: nutrient composition and health benefits. Working Paper 67. Internationl Food Policy Research Institute. 1-17

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem, 239(1), 70-76. doi: 10.1006/abio.1996.0292

Bultosa G. (2007). Physicochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc.) Trotter] grain varieties. J. Appl. Sci. Res. 3 2042–2051.

Dehghan-Shoar, Z., Hardacre, A. K., & Brennan, C. S. (2010). The physico-chemical characteristics of extruded snacks enriched with tomato lycopene. Food Chemistry, 123(4), 1117-1122. doi: https://doi.org/10.1016/j.foodchem.2010.05.071

Deng, G.-F., Xu, X.-R., Guo, Y.-J., Xia, E.-Q., Li, S., Wu, S., . . . Li, H.-B. (2012). Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. Journal of Functional Foods, 4(4), 906-914. doi: https://doi.org/10.1016/j.jff.2012.06.008

Dykes, L. and Rooney, W. (2007) Phenolic Compounds in Cereal Grains and Their Health Benefits. Cereal Foods World, 52, 105-111. doi:10.1094/CFW-52-3-0105

Dlamini, N. R., Taylor, J. R. N., & Rooney, L. W. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. Food Chemistry, 105(4), 1412-1419. doi: https://doi.org/10.1016/j.foodchem.2007.05.017

Eerlingen, R. C., & Delcour, J. A. (1995). Formation, analysis, structure and properties of type III enzyme resistant starch. Journal of Cereal Science, 22(2), 129-138. doi: https://doi.org/10.1016/0733-5210(95)90042-X

European Commission, 2006. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union L404, 9e25.

Forsido, S. F., Rupasinghe, H. P. V., & Astatkie, T. (2013). Antioxidant capacity, total phenolics and nutritional content in selected ethiopian staple food ingredients. International Journal of Food Sciences and Nutrition, 64(8), 915-920. doi: 10.3109/09637486.2013.806448

Foschia, M., Peressini, D., Sensidoni, A., & Brennan, C. S. (2013). The effects of dietary fibre addition on the quality of common cereal products. Journal of Cereal Science, 58(2), 216-227. doi: http://dx.doi.org/10.1016/j.jcs.2013.05.010

Friedman, M. (1996). Food Browning and Its Prevention: An Overview. Journal of Agricultural and Food Chemistry, 44(3), 631-653. doi: 10.1021/jf950394r

Gebremariam, M. M., Zarnkow, M., & Becker, T. (2014). Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. Journal of Food Science and Technology, 51(11), 2881-2895. doi: 10.1007/s13197-012-0745-5

Gökmen, V., Serpen, A., & Fogliano, V. (2009). Direct measurement of the total antioxidant capacity of foods: the 'QUENCHER' approach. Trends in Food Science & Technology, 20(6), 278-288. doi: https://doi.org/10.1016/j.tifs.2009.03.010

Gumul D., Korus J. Polyphenol content and antioxidant activity of rye bran extrudates produced at varying parameters of extrusion process. *Food Science and Technology*. 2006;9(4):1–11.

Guo, W., & Beta, T. (2013). Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. Food Research International, 51(2), 518-525. doi: https://doi.org/10.1016/j.foodres.2013.01.008

Hager, A.-S., Wolter, A., Jacob, F., Zannini, E., & Arendt, E. K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56(2), 239-247. doi: https://doi.org/10.1016/j.jcs.2012.06.005

Holguín-Acuña, A. L., Carvajal-Millán, E., Santana-Rodríguez, V., Rascón-Chu, A., Márquez-Escalante, J. A., Ponce de León-Renova, N. E., & Gastelum-Franco, G. (2008). Maize bran/oat flour extruded breakfast cereal: A novel source of complex polysaccharides and an antioxidant. Food Chemistry, 111(3), 654-657. doi: https://doi.org/10.1016/j.foodchem.2008.04.034

Ibanoglu, S., Ainsworth, P., Ozer, E. A., & Plunkett, A. (2006). Physical and sensory evaluation of a nutritionally balanced gluten free extruded snack food. Journal of Food Engineering, 75, 469-472. doi:10.1016/j.jfoodeng.2005.04.060

Ilo, S., Liu, Y., & Berghofer, E. (1999). Extrusion Cooking of Rice Flour and Amaranth Blends. LWT - Food Science and Technology, 32(2), 79-88. doi: http://dx.doi.org/10.1006/fstl.1998.0497

Inglett, G. E., Chen, D., & Liu, S. X. (2016). Physical Properties of Gluten Free Sugar Cookies Containing Teff and Functional Oat Products. Journal of Food Research; Vol 5, No 3 (2016). doi: 10.5539/jfr.v5n3p72

Kim, J. H., Tanhehco, E. J., & Ng, P. K. W. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. Food Chemistry, 99(4), 718-723. doi: https://doi.org/10.1016/j.foodchem.2005.08.054

Kotásková, E., Sumczynski, D., Mlček, J., & Valášek, P. (2016). Determination of free and bound phenolics using HPLC-DAD, antioxidant activity and in vitro digestibility of

Eragrostis tef. Journal of Food Composition and Analysis, 46, 15-21. doi: https://doi.org/10.1016/j.jfca.2015.11.001
Laus MN, Tozzi D, Soccio M, Fratianni A, Panfili G, Pastore D (2012) Dissection of antioxidant activity of durum wheat (Triticum durum Desf.) grains as evaluated by the new LOX/RNO method. J Cereal Sci 56:214–222
Mäkilä, L., Laaksonen, O., Ramos Diaz, J. M., Vahvaselkä, M., Myllymäki, O., Lehtomäki, I., . . . Kallio, H. (2014). Exploiting blackcurrant juice press residue in extruded snacks. LWT - Food Science and Technology, 57(2), 618-627. doi: https://doi.org/10.1016/j.lwt.2014.02.005
McGee H (2004). On food and cooking (First Scribner Revised Edition). NY: Scribner
Mkandawire, N. L., Weier, S. A., Weller, C. L., Jackson, D. S., & Rose, D. J. (2015). Composition, in vitro digestibility, and sensory evaluation of extruded whole grain sorghum breakfast cereals. LWT - Food Science and Technology, 62(1), 662-667. doi: http://dx.doi.org/10.1016/j.lwt.2014.12.051
Mora-Rochin, S., Gutiérrez-Uribe, J. A., Serna-Saldivar, S. O., Sánchez-Peña, P.,Reyes-Moreno, C., Milán-Carrillo, J. 2010. Phenolic content and antioxidant activity of tortillas produced from pigmented maize processed by conventional nixtamalization or extrusion cooking JOURNAL OF CEREAL SCIENCE52, 3; 502-508;
O″zer, E. A., Herken, E. N., Güzel, S., Ainsworth, P., & İbanoğlu, Ş. (2006). Effect of extrusion process on the antioxidant activity and total phenolics in a nutritious snack food. *International Journal of Food Science & Technology, 41*(3), 289-293. doi: 10.1111/j.1365-2621.2005.01062.x
Obrenovich, M. E., Nair, N. G., Beyaz, A., Aliev, G., & Reddy, V. P. (2010). The role of polyphenolic antioxidants in health, disease, and aging. *Rejuvenation Res, 13*(6), 631-643. doi: 10.1089/rej.2010.1043
Oliveira, L. C., Rosell, C. M., & Steel, C. J. (2015). Effect of the addition of whole-grain wheat flour and of extrusion process parameters on dietary fibre content, starch transformation and mechanical properties of a ready-to-eat breakfast cereal. *International Journal of Food Science & Technology, 50*(6), 1504-1514. doi: 10.1111/ijfs.12778
Omwamba, M., & Hu, Q. (2010). Antioxidant Activity in Barley (Hordeum Vulgare L.) Grains Roasted in a Microwave Oven under Conditions Optimized Using Response Surface Methodology. *Journal of Food Science, 75*(1), C66-C73. doi: 10.1111/j.1750-3841.2009.01426.x
Pastore D, Laus MN, Tozzi D, Fogliano V, Soccio M, Flagella Z (2009) New tool to evaluate a comprehensive antioxidant activity in food extracts: bleaching of 4-nitroso-N, N-dimethylaniline catalyzed by soybean lipoxygenase-1. J Agric Food Chem57:9682–9692
Pokorný, J., & Schmidt, Š. (2001). 14 - Natural antioxidant functionality during food processing *Antioxidants in Food* (pp. 331-354): Woodhead Publishing.
Potter, R., Stojceska, V., & Plunkett, A. (2013). The use of fruit powders in extruded snacks suitable for Children's diets. *LWT - Food Science and Technology, 51*(2), 537-544. doi: http://dx.doi.org/10.1016/j.lwt.2012.11.015
Ramos Diaz, J. M., Suuronen, J.-P., Deegan, K. C., Serimaa, R., Tuorila, H., & Jouppila, K. (2015). Physical and sensory characteristics of corn-based extruded snacks containing amaranth, quinoa and kañiwa flour. *LWT - Food Science and Technology, 64*(2), 1047-1056. doi: https://doi.org/10.1016/j.lwt.2015.07.011

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.

Remy S, Fulcrand H, Labarbe B, Cheynier V, Moutounet M 2000. First confirmation in red wine of products resulting from ditect anthocyanin-tannin reactions. Journal of the Science of Food and Agriculture, 80: 745-751.

Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods, 5*(2), 570-581. doi: https://doi.org/10.1016/j.jff.2013.02.004

Sharma, P., Gujral, H. S., & Singh, B. (2012). Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry, 131*(4), 1406-1413. doi: https://doi.org/10.1016/j.foodchem.2011.10.009

Singh, B., Sekhon, K. S., & Singh, N. (2007). Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. *Food Chemistry, 100*(1), 198-202. doi: https://doi.org/10.1016/j.foodchem.2005.09.042

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299: 152–178.

Società Italiana di Nutrizione Umana. (2014), *Livelli di Assunzione di Riferimento di Nutrienti ed energia per la popolazione italiana IV revision*. SICS, Italy.

Solomon, W. K. (2014). Hydration kinetics of direct expanded tef flour breakfast cereals in water and milk. *Food Science & Nutrition, 2*(1), 39-45. doi: 10.1002/fsn3.80

Stojceska, V., Ainsworth, P., Plunkett, A., & İbanoğlu, Ş. (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chemistry, 114*(1), 226-232. doi: http://dx.doi.org/10.1016/j.foodchem.2008.09.043

Stojceska, V., Ainsworth, P., Plunkett, A., & İbanoğlu, Ş. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry, 121*(1), 156-164. doi: http://dx.doi.org/10.1016/j.foodchem.2009.12.024

Symons, L. J., & Brennan, C. S. (2004). The Influence of (1→3) (1→4)-β-D-Glucan-rich Fractions from Barley on the Physicochemical Properties and In Vitro Reducing Sugar Release of White Wheat Breads. *Journal of Food Science, 69*(6), C463-C467. doi: 10.1111/j.1365-2621.2004.tb10989.x

Tufan, A. N., Çelik, S. E., Özyürek, M., Güçlü, K., & Apak, R. (2013). Direct measurement of total antioxidant capacity of cereals: QUENCHER-CUPRAC method. *Talanta, 108*, 136-142. doi: https://doi.org/10.1016/j.talanta.2013.02.061

Viscidi, K. A., Dougherty, M. P., Briggs, J., & Camire, M. E. (2004). Complex phenolic compounds reduce lipid oxidation in extruded oat cereals. *LWT - Food Science and Technology, 37*(7), 789-796. doi: https://doi.org/10.1016/j.lwt.2004.03.005

Vitaglione, P., Napolitano, A., & Fogliano, V. (2008). Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science & Technology, 19*(9), 451-463. doi: https://doi.org/10.1016/j.tifs.2008.02.005

Wang, T., He, F., & Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods, 7*, 101-111. doi: https://doi.org/10.1016/j.jff.2014.01.033

Wang, Y.-Y., & Ryu, G.-H. (2013). Physicochemical and antioxidant properties of extruded corn grits with corn fiber by CO2 injection extrusion process. *Journal of Cereal Science, 58*(1), 110-116. doi: https://doi.org/10.1016/j.jcs.2013.03.013

Whalen, P.J. (2009). Extruded products and degree of cook. In: The RVA handbook, Crosbie GB, Ross AS, AACC International, St. Paul (MN, USA).

Zhang, M., Bai, X., & Zhang, Z. (2011). Extrusion process improves the functionality of soluble dietary fiber in oat bran. *Journal of Cereal Science, 54*(1), 98-103. doi: https://doi.org/10.1016/j.jcs.2011.04.001

Zhu, F. (2018). Chemical composition and food uses of teff (Eragrostis tef). *Food Chemistry, 239*(Supplement C), 402-415. doi: https://doi.org/10.1016/j.foodchem.2017.06.101

Zilic, S., Mogol, B. A., Akillioglu, G., Serpen, A., Delic, N., & Gokmen, V. (2014). Effects of extrusion, infrared and microwave processing on Maillard reaction products and phenolic compounds in soybean. *J Sci Food Agric, 94*(1), 45-51. doi: 10.1002/jsfa.6210

CHAPTER 6. Cereal-based and insect-enriched printable food: from formulation to post processing treatments. Status and perspectives.

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Abstract
3D printing is a layer-by-layer process capable to manufacture new objects from several materials, among which food formulation. In the field of cereal-based products it has the potential to make objects having very complex shapes. This intricate structure could lead to the obtaining of new textural properties of the end-products never thought before. However, most of cereal-based 3D printed objects need post-processing treatments, such as cooking, frying, baking, etc., which could cause the collapse of the printed structure. The use of new ingredients and binding agents would be of relevant importance in this technology to obtain innovative food product and to keep the designed structure. Among these, insects have a potential in the field of cereal-based products due to their high content in protein. Because of the skepticism in the Western countries, the use of 3D printing could be useful to improve shape, taste and in turn their acceptability.

The aim of this chapter is to evaluate current researches in the field of cereal-based 3D printed products. We will review knowledge on 3D printing methods, printing conditions, possibility to improve the printability, compatibility with traditional food processing technologies, possibility to obtain innovative healthy food enriched with insects

Keywords: teff, 3D Printing, cereal-based products, insect-enriched products, post-processing technologies, printability, innovative food formulation

6.1 Introduction

Additive Manufacturing (AM), widely known as 3D printing, is an emerging technology for direct fabrication that involves the layer-by-layer deposition of materials to form a 3D structure which may not be achievable with traditional techniques. It is leading to a revolution across many sectors and it is experiencing a wide range of application that would have not been even considered feasible until a few years ago (Balletti et al., 2017). In the last ten years it has been used in food science for the fabrication of new functional products or for the production of new shapes, texture, flavour, colour, etc. In addition, the design and the development of 3D edible objects with modified nutritional compounds are also possible with this technology. With the increasing interest in healthy food, there is a constant attention for innovative methods of food processing to address people with various medical problems or with special dietary needs (Hamilton et al., 2018).

The reduction in size and costs of 3D printers favoured and contributed to the growth of this technology as well as its commercialization (Balletti et al., 2017).

Some of the benefits of 3D printing technology can be summarized as follow: 1. Automated manufacturing; 2. Ability to produce custom products at relatively low costs; 3. High range of application and products; 4. No or low waste material; 5. Speed and ease of designing (Berman, 2012).

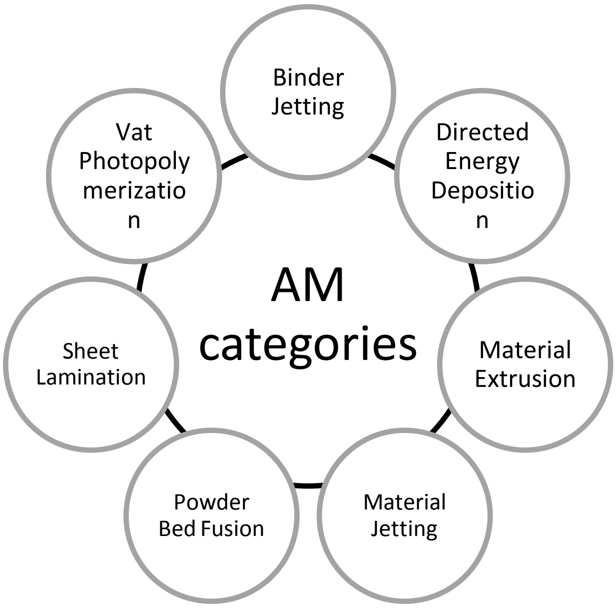
Over the past few years numerous research studies have been performed on 3D printing, exploring the use of different food materials, for example chocolate (Hao et al., 2010), cereal-based products (Lipton et al., 2015; Severini et al., 2016), cheese (Le Thoic et al., 2018; Periard et al., 2007), fruits and vegetable products (Derossi et al., 2018; Severini et al., 2018), etc.

In this chapter we want to analyze the most important advances of the application of 3D printing for cereal-based products and insect-enriched products. In particular paragraph 6.2 is dedicated to the knowledge of the different techniques used in 3D cereal products, paragraph 6.3 investigates the effects of ingredients and binding agents on the printability of 3D objects. Also, the effect of the most used methods for cooking cereal based products (paragraph 6.4), textural properties (paragraph 6.5) and the effect of some printing variables affecting the quality of 3D structures (paragraph 6.6) are explored. In paragraph 6.7 the possibility to obtain innovative cereal based products with 3D printing is examined. In particular, the use of edible insect in 3D food printing is investigated, with the aim to enrich cereal products.

6.2 3D-printing technologies for cereal-based formulation.

Since the AM technology has been developed, several techniques were born. Recently the International Organization for Standardization (ISO)/American Society for Testing Materials (ASTM) have classified seven process categories (Figure 6.1) such as powder bed fusion, directed energy deposition, material jetting, binder jetting, material extrusion, vat polymerization and sheet lamination (ASTM International, 2015).

Figure 6.1 Categories of AM processes based on ASTM International (2015).



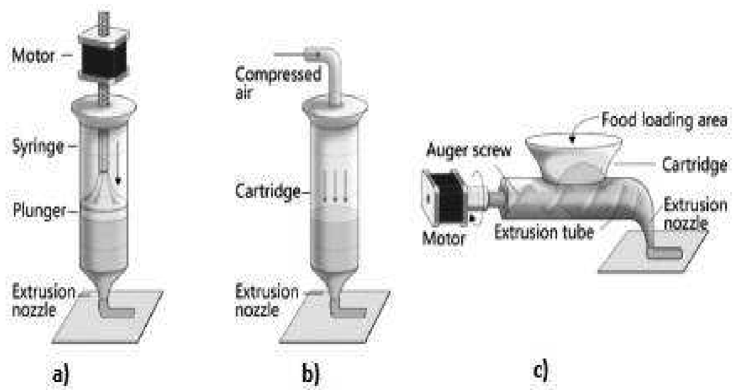
All these categories consist in several sub-technologies too, each of which have similarities as well as differences. Most of the above 3D printing technologies have been used in several field such as aerospace, medical and pharmaceutical applications, engineering, etc. Considering the application in food sector, not all foods are compatible with every 3D printing technique. From this, the need to accurately choose the technology in respect to the kind of food formulation and the end-product is evident.

Regarding the field of cereals, two main technologies have been widely applied: material extrusion and powder bed fusion. The extrusion-based 3D printing was firstly introduced by

Crump with his method Fused Deposition Model (FDM) (Crump, 1991). This method was initially used for prototyping plastic or metal, but now it is used in the food sector too. In the extrusion process a molten or semi-solid filament of food ingredients is extruded out of a moving nozzle by the force produced by a hydraulic piston, by the pressure caused from a compressor or by an auger screw (Figure 6.2). The nozzle tip moves in x- and y- directions while the platform moves down in z- direction. The movements of the nozzle enable to built, layer by layer, a 3D virtual model previously designed by CAD software.

The binding mechanisms between layers, that allow the creation of a 3D object, involve the rheological properties of the materials, the solidification upon cooling and the hydro gel-forming extrusion too (Godoi et al., 2016). The extrusion based 3D food printing has some advantages which are: automation of the food preparation process, improvement of process efficiency and final food quality, low maintenance cost, low waste material, personalization of shape and dimension, possibility to use alternative and new ingredients. As opposite it presents limited material choices and, sometimes, long fabrication time (Sun et al., 2015). In this kind of 3D technique the conventional food extrusion process is completely digitalised and the manipulation of the food fabrication can occur in real time (Sun et al., 2018).

Figure 6.2 Material extrusion mechanisms (Sun et al., 2018). a) Syringe-Based Extrusion, b) Air Pressure Driven Extrusion, c) Screw-Based Extrusion.

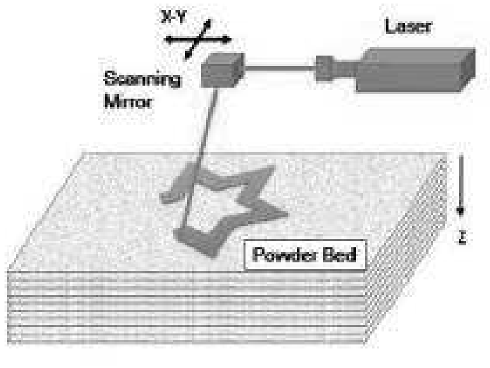


By this process several 3D cereal-based products have been developed. Lipton et al. (2010) fabricated modified traditional cookies with a novel and more stable recipe by changing the conventional ratio of the ingredients. The same authors (Lipton et al., 2015) printed a pizza in 30 minutes with a air pressure driven extrusion system, by depositing a dough added with water and a sauce added with xanthan guar as thickener. Recently the Netherlands Organisation for Applied Scientific Research (TNO) collaborated with the world's leading pasta maker, Barilla spa, with the aim to produce new shapes of pasta. In 2015 they presented some printed pasta shapes obtained from traditional ingredients, such as durum wheat and water (Van der Linden, 2015).

However some other studies were performed in order to obtain cereal-based products (Lille et al., 2018; Severini et al., 2016) by this process, while only one example was achieved with a different kind of 3D technique, such as Selective Laser Sintering (SLS) (Agerawi et al., 2015). SLS is the second most popular method in 3D food printing (Godoi et al., 2016) and it is a sub-type of the big category of powder binding fusion. It uses a laser as a sintering source of powdered food material. As visible in Figure 6.3, the laser is conducted by a scanning mirror in a point of the space pre-defined by a 3D model in order to fuse together the layers of particles and to create a solid structure through sintering (Balletti et al., 2017). At the end of the process an additional step is necessary for removing the unfused material.

This technique presents some advantages, such as the use of several materials and no support structures requirements. Furthermore this technique well fit with the production of a multiple layers of food matrix, each of these containing different food ingredients (Diaz et al., 2014). SLS had been extensively used with metals, ceramic, etc., but now it is being adopted in the food sector, especially to print sugar structures.

Figure 6.3 Schematic representation of the Selective Laser Sintering technology (Sun et al., 2015)



When heat is used as power source the end products have low nutritional value, and for this reason toughness and uniform surface of the products become relevant characteristics of the 3D object (Godoi et al., 2016).

As above reported Agerawi et al., (2015) printed with SLS technique some cookies made with different flour type, such as semolina, soft wheat flour and a mix of flour and starch. Moreover they used different ratios of structure and binder and different binder ratios. Respect to traditional backed cookies, the printed ones had smaller average wall thickness. The use of semolina and soft flour wheat led to thicker walls, rather than with a flour/starch mixture. Finally the structure: binder ratio had a negligible effect on wall thickness, while the binder composition of 90:10 leads to significantly thicker walls.

In the next future the ability to obtain cereal-based products with other 3D process could be of high interest.

6.3 Factors affecting the printability of a dough

Printability is defined as 'the property of a material which allow to support its own weight and to retain the structure' (Godoi et al., 2016). It is an important parameter in 3D printing, which is related with the viscosity and the rheological properties of the food matrix. Some factors can affect printability of a food filament, first of all the ingredient used and their interaction, as well as some binding agents, which have a fundamental role in changing the rheological behavior of a mixture.

The effects of these compounds and of the ingredients will be well discussed in the following sections.

6.3.1 Effect of ingredients and nutritional compounds in the 3D-printing process

Printable food formulations are generally composed of several ingredients, that affect viscosity, stability and printability of the formulation differently. The stability of a 3D edible object is related to the printability of the food formula, depending on the kind and the ratios of all ingredients and their physical properties.

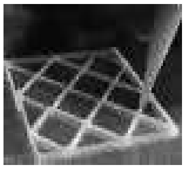
In the study of Kim et al. (2018) the authors explored the printability of some reference materials by increasing the concentration of hydrocolloids and, in addition, some experimental foods. In particular, they investigated the dimensional stability, the shear modulus and the handling properties of 3D printed food to analyze the printability of the materials. While the results of the reference materials were similar for all the investigated parameters, the printed food showed many differences. The reason of such discrepancy was probably due to the fact that the reference materials contained only one element, while food materials consist in multiple ingredients and components with unique properties and different constituent interaction (Kim et al., 2018). For example they classified cookies dough as difficult to extrude, based on its rheological properties and mechanical specifications. They concluded that the dough for cookies required high output extruder for printing enabling the printing of only simple models with low height, because the dough structure does not allow retaining its shape (Kim et al., 2018).

Lipton et al. (2010) modified the ratio of the ingredients for cookies in order to enable the 3D printing of cookie recipe. In particular they founded that increasing the amount of butter the shape stability after printing decreased, while adding more egg yolk to the formulation the width/length stability increased and the height stability decreased. This was probably due to the lower viscosity of the egg yolk- based dough than to the butter-based dough, which led to a flatten of the printed filaments and, as a results, they obtained a width and short 3D object.

Garcia-Julia et al. (2015) studied the effects of beef burger, cheese cracker dough and pizza dough, during 3D printing. Regarding the effect of the ingredients, they founded that increasing amount of salt and pepper caused the solubilization of the myofibrillar proteins in the beef burger, leading to a decrease of the force required for the extrusion. Probably also in other protein-based products salt and pepper could have the same effect by modifying the pH and, in turn, causing the partial denaturation of the proteins.

Regarding fat, a poor interaction between layers caused the presence of empty spaces between layers and this could provoke fractures in the final object and the undesirable migration of fat in the empty channels (Godoi et al., 2016). With the aim to better evaluate the role of fats in the 3D printing process, Lille et al. (2017) investigated the effects of both skimmed milk powder (SMP) and semi-skimmed milk powder (SSMP) on the viscosity of printed samples. SMP at a solid content of 50% formed a high-viscosity paste, which resulted in an irregular deposition of materials. Instead increased concentration of SMP to 60% improved the viscosity of the paste making possible the printing. On the contrary the SSMP paste was well printed at a concentration of 60% and the shape was kept very well also after printing (Figure 6.4). Probably the higher fat content of SSMP respect to SMP, which was of 9 and 0.4% respectively, was responsible of plasticization or lubrication effect making the material more easily flowing (Lille et al., 2018).

Figure 6.4 Printability of pastes prepared from milk powder (from Lille et al., 2018)

60% Skimmed Milk Powder	
60% Semi-Skimmed Milk Powder	

However the fundamental importance of the ingredients on the printability of the food material is based on the constituents of the food matrix. A better understanding of effect of the principal nutritional compounds of foods on the AM technology is crucial in order to obtain a stable 3D object and to guarantee the quality of the end-products. Godoi et al. (2016) explored the effect of the essential constituents of foods: carbohydrates, proteins and fats. Regarding carbohydrates it is important the control of the glass transition temperature (Tg) to deposit the material and to support its own weight after deposition. Simple sugars have low Tg values while high molecular weight carbohydrates, such as maltodextrins or starch, have high Tg. These latter carbohydrates are unprintable without prior modification before printing process, so they need addition of water or gelling agent or heat, in order to broken down the intermolecular bonds of starch and to depress the Tg. On the other hand, increasing starch content in a food formula intensified the closer linkage between water and starch, leading to the formation of a denser network structure and, in turn, increasing the viscosity (Liu et al., 2018). Liu et al. (2018) demonstrated that adding 4% of potatoes starch to mashed potatoes, the printed object showed a good shape retention but poor resolution and large deviation from the target constructs. This was probably due to the too high viscosity resulted in the poor printability (Liu et al., 2018).

Proteins, because composed of amino acids, contained positive and negative charged groups. The application of external stress, such as temperature or mechanical strength, or strong acid or base compounds, caused the aggregation or denaturation of proteins, which could significantly affects the viscosity of the materials.

Anyway the most important factor affecting the material formulation for AM technology and the end-use properties of the material is the triglyceride composition. For example the triglyceride composition regulates the melting point of the deposited layers and, so, the self-supporting properties of the food materials. In particular fatty acids with larger number of carbon atoms depicts higher melting point, while larger number of double bonds results in lower melting points (Godoi et al., 2016).

These data could be useful for the correct development of a food formulation suitable in a 3D printing process, but additional knowledge is necessary for a better performance of this technology, especially for cereal-based products.

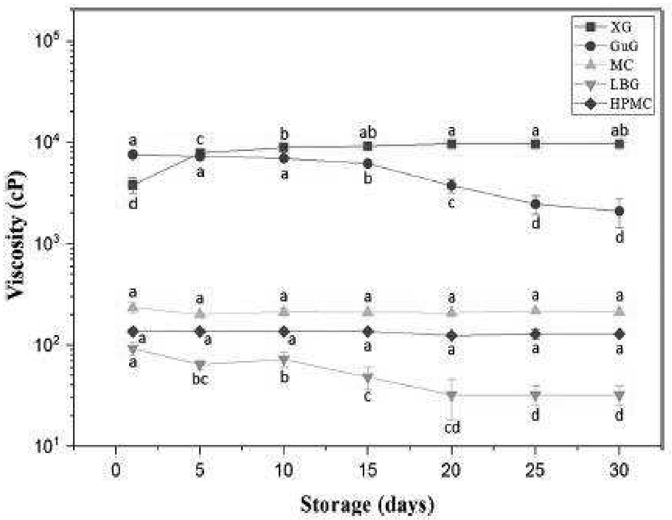
6.3.2 The use of binding agents to improve the printability of dough.

The printability is highly related to the rheological properties of the materials. The viscosity of materials has to be low enough to allow the flow through the nozzle, but it needs to be also high enough for supporting the structure after deposition and to adhere to previous deposited layer (Lille et al., 2018). Sometimes food materials need to be mixed with additives in order to change and adjust the rheological properties of the printed dough and to be compatible with printing process, especially in Fused Deposition Model. Generally hydrocolloids are used as rheological modifiers within food safety standards (Liu et al., 2018) and they are often added in foods, like rice, meat, fruits and vegetables, which are non-printable by nature (Sun et al., 2015). Hydrocolloids, such as xanthan gum, gelatin and agar, are hydrophilic polymers that are used as thickening and gelling agents for water-based solutions (Kim et al., 2018). Cohen et al. (2009) used hydrocolloids with the aim to improve the printability and texture of their food material. In particular, they tried to create a wide range of mouthfeel matrix by adding two hydrocolloids, xanthan gum and gelatin, in different concentrations and combinations. The study showed as, by adding 0.5% of gelatin, a milk-like texture was obtained, while increasing the concentration of gelatin at 4% they obtained a mushroom-like texture. Another interesting result was that combining the two hydrocolloids the food material started to possess granularity which augmented with increasing the xanthan:gelatin ratio (Cohen et al., 2009).

Kim et al. (2018) used hydrocolloids as reference materials to assess the printability of foods, which have been analyzed by testing the dimensional stability and the handling properties. They investigated 7 kinds of hydrocolloids, and, as a result they selected methylcellulose as the most suitable hydrocolloids as a reference material, due to its versatility and its rheological stability. As observed in Figure 6.5, methylcellulose showed the highest stability in viscosity and the best texture attributes in comparison with the other investigated hydrocolloids until 30 days of storage at 25°C (data not shown) (Kim et al., 2018).

These hydrocolloids, as well as microcrystalline cellulose or carboxymethylcellulose, are used as bulk filling agents in food formulations and they are highly used in the food industry because of their low cost and huge availability (Holland et al., 2018). Also cellulose nanofiber was used in 3D printing to improve shape stability of the printed structure (Lille et al., 2018). In addition, the concentration of the reference material was directly proportional with the shear modulus values, which is a factor that well characterizes the rheological properties and the dimensional stability, so the printability of a food material (Kim et al., 2018).

Figure 6.5 Viscosity variation of 5 hydrocolloids during 30 days of storage at 25°C (Kim et al., 2018) XG: xanthan gum; MC: methylcellulose; LBG: locus bean gum; HPMC: hydroxypropyl methylcellulose



Xanthan gum is another polysaccharide frequently used in food industry, because at low concentrations exhibits a very high viscosity of solution (Holland et al., 2018). Lipton et al. (2015) added xanthan gum in a pizza-sauce to act as a thickener. The addition of small amount of both xanthan gum and cellulose, can reinforce binding mechanisms, so they could be favorable in the ink jetting printing. They can act synergistically and can create a particle network in order to realize a stabile 3D structure (Hollan et al., 2018).

Lipton et al. (2010) examined the use of two food additives, transglutaminase and agar, to obtain printable food material. In both cases the use of these additives led to the creation of complex geometries of foods and increased the dimensional stability of the 3D printed objects (Lipton et al., 2015). In addition, varying additive concentration, new texture, new tastes and material strengths will be produced (Lipton et al., 2010). In figure 6.6 images of three complex geometries of 3D printed object obtained with the addition of transglutaminase to scallop and turkey are shown. These images proved as transglutaminase allows the food material to retain the shape, with special emphasis to the curves, edges and vertical surface.

Figure 6.6 Images of 3D printed objects obtained from the addition of transglutaminase to scallop a), b) and to turkey c) (modified from Lipton et al., 2015 and from Lipton et al., 2010).

a) **b)** **c)**

Wancauwenberghe et al. (2015) studied pectin-based formulations with the aim to print edible objects, which were then incubated in a $CaCl_2$ solution in order to solidify the structure. They found that a too low $CaCl_2$ concentration could cause the swelling of the printed object while a too high $CaCl_2$ concentration caused an irregular deposition of the pectin-gel filament as well as a non-complete adhesion of the layers.

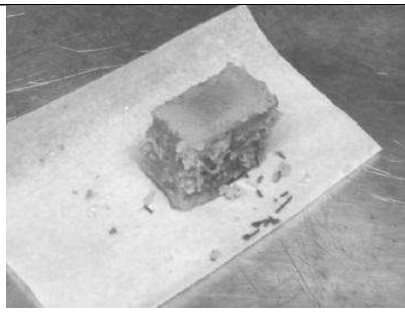
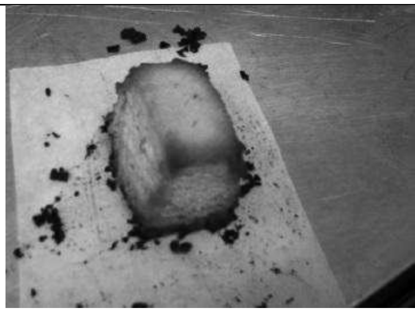
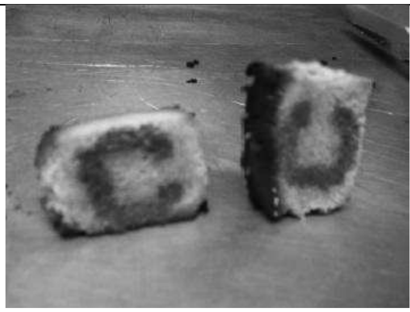
Another additive used for its functionality as a thickening/gelling agent was starch. It helps to retain the shape after deposition and facilitates the extrusion of the food material through the nozzle (Lille et al., 2018).

From the previous discussion it is possible to conclude that hydrocolloids are useful in 3D printing technology because of their capabilities to act as gelling agents, to improve the rheological characteristics of a food formula and to increase the printability of a dough. Even if none of these molecules have been applied in cereal-based products, hydrocolloids are generally used in water-based solution. Thus, since dough is usually prepared by mixing flour and water, hydrocolloids could also be used for example in low starch cereals, which present a poor viscosity.

6.4 Post-processing technologies.

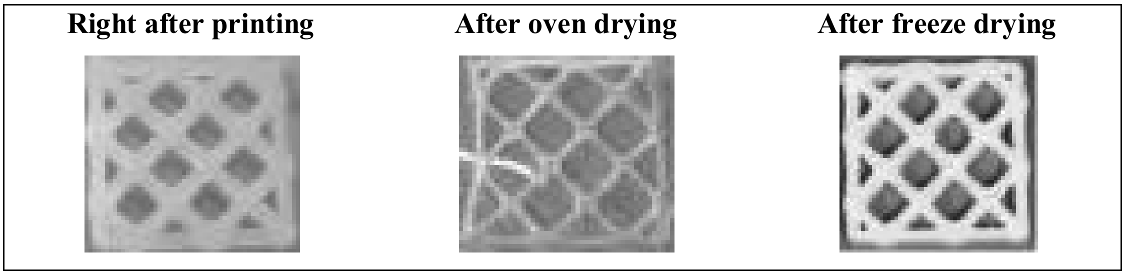
In 3D food printing, especially with cereal-based dough, a post-deposition cooking process is often required, such as baking, steaming, frying, etc. During the cooking process many chemical reactions, such as Maillard reactions, protein denaturation, water content reduction and physical transformations (for example change in color, volume, texture) can occur (Sun et al., 2018). As an ideal opportunity, 3D printers should integrate the process of printing with the cooking after deposition, which are currently separated. In addition, some researchers hypothesized alternative methods to use in the future for the post-deposition cooking. For example, they suggest an instant infrared radiation to substitute the baking process (Sun et al., 2018). To date, none of these methods are possible, so one of the most important challenges in 3D printing is to maintain the 3D shape throughout the post-cooking processes. Some studies were carried out in order to investigate the effects of the post processing on 3D printed edible objects. Lipton et al. (2010) printed a modified traditional cookie recipe and studied the shape retention after baking. In figure 6.7 is possible to examine as the exterior of the backed cookie slumped but the interior geometry was successfully preserved (Lipton et al., 2010).

Figure 6.7 Images of a 3D printed cookies before and after baking (modified from Lipton 2010)

Before baking		
After baking	**Exterior view**	
	Interior view	

Lille et al. (2018) investigated the effects of two drying method on some printed pastes composed by a mixture of protein, starch and fiber-rich food ingredients. They pointed out that freeze-drying better preserved the printed structure than oven drying at 100°C, as it is well visible in Figure 6.8.

Figure 6.8 Example of 3D printed sample after printing and after freeze-drying and oven drying processes (modified from Lille et al., 2018).



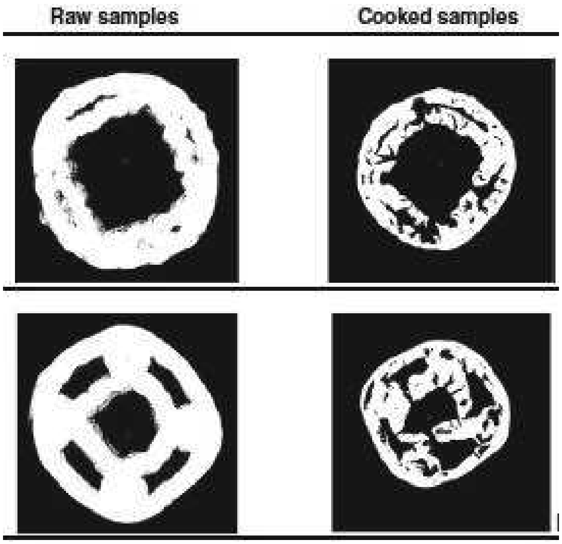
During the freeze-drying process the material is dehydrated in frozen state and the structure well resisted to the stress, while during the conventional oven drying the hot air applied led to the shrinkage of the samples (Ratti, 2001; Mayor & Sereno, 2004). An interesting result was that, applying both drying methods to samples with a dry matter content of at least 45%, the same retention of the structure was obtained. This was probably due to the lower amount of water removed and a possible initial higher structural strength (Lille et al., 2018).

Severini et al (2016) studied the changes in dimension, shape, microstructure and texture properties of wheat-based food products. In this study they observed as the experimental diameter of samples after cooking decreased as an effect of the water removal. The differences among raw and cooked samples are shown in Figure 6.9, which is an X-ray microCT image of the transversal section of two wheat-based 3D printed samples.

Analyzing the effects of cooking on microstructure of samples, the authors pointed out that experimental data of the solid fraction of cooked samples were lower than uncooked sample ones. This effect was due to the vapor produced during cooking which increased the dimension of the pre-existing pores and created new ones.

Post-processing is, in some cases, an important step in a 3D printing process. An accurate selection of materials and of their physical-chemical, rheological and mechanical properties are of utmost importance to develop 3D food objects, which can resist to post-processing conventional methods.

Figure 6.9 X-ray microCT images of transversal section of two wheat-based 3D printed samples (adapted from Severini et al., 2016)



6.5 Textural properties of 3D printed cereal-based products.

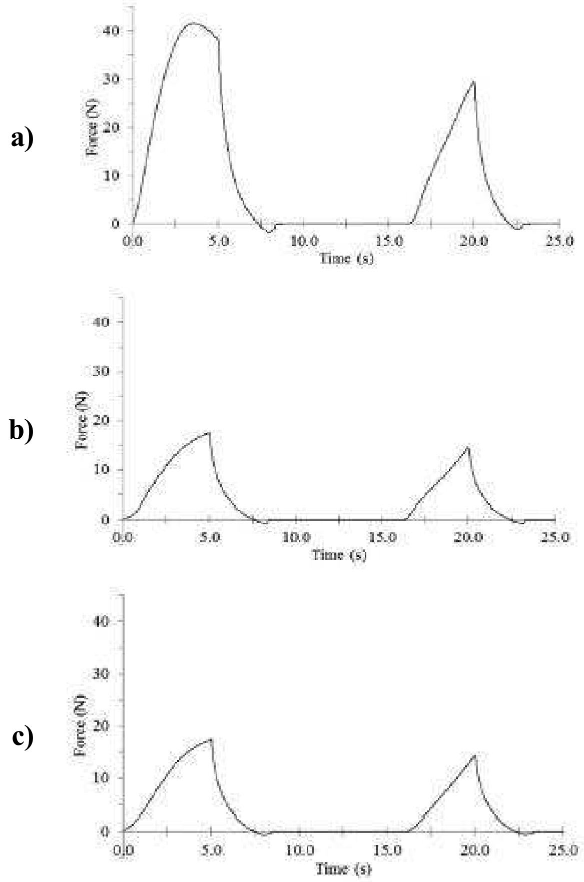
The term texture refers to those quality of a food that can be felt by fingers, tongue, palate or teeth, which are able to distinguished foods by each other, but it is also one of the most important attributes used by consumers to assess food quality (McKenna, 2003). For example stored bread become hard and stale while fruits and vegetables lose their turgor pressure with long storage period, due to the water loss. So the evaluation of texture properties of foods is essential for determining the sensorial characteristics as perceived in the mouth. In food texture testing, some standard tests are used, such as compression, tension and flexure, in order to measure hardness, crispiness, crunchiness, softness, springiness and some other attributes of food products. In processed foods the analysis of textural properties can be used for optimizing the process conditions.

Regarding the effects of the ingredients used in the food formula and of the post-processing treatments on textural attributes, Lille et al. (2018) investigated the hardness of four different protein-enriched 3D samples, right after two drying methods. They showed as semi-skimmed milk powder samples have the highest hardness after oven drying, while the fava bean protein concentrate samples showed the highest hardness after freeze-drying. In this study the authors demonstrated as the optimization of both food formula and drying method could lead to develop a product with desired mechanical properties.

In 3D printing technology some printing variables could affect the texture of the end products. As an example, the breaking strength of some wheat-based products decreased when reducing the infill level, even if a high variance between samples was observed (Severini et al., 2016). Also another study investigated the effects of the printing process as well as of the extrusion speed during printing on the hardness of printed cheese. The authors found a significant decrease of hardness of printed samples respect to the untreated ones, while the extrusion speed showed no impact on texture (Figure 6.10) (Le Tohic et al., 2018). Even if this study was not concerning on cereal products it could be however interesting for the understanding of the effect of 3D technology on texture properties of printed samples.
Thus, texture could change by printing food materials: especially with the extrusion process, when the structure of the materials is partially disrupted. Moreover, texture could be controlled by printing together materials with different texture or by printing the same materials in different voids fractions which result into a different texture. Another alternative method to change the texture is to print the food material into a grid structure; in this way the contact points between strands of materials are reduced, so the air could easily infiltrate into the
structure (Batchelder, 1995). As an example, a corn dough was 3D printed with lots of voids inside the structure becoming high porous. At the end of the printing process the corn dough consisted of a strand like network (Lipton & Lipson, 2011).

In the next future the production of new texture from foods would be possible by making food material printable through the changing of the void content, of the structure of materials and by blending multiple materials together (Lipton et al., 2015).

Figure 6.10 Texture profile analysis of untreated samples a), samples printed at low speed b), samples printed at high speed c) (modified from Le Tohic et al., 2018)



6.6 Printing variables affecting the quality of cereal-based products.

In the 3D printing, as well as in all the other technologies, the quality of the end products is also determined by process parameters. For example, in the extrusion cooking technology, the screw speed, the barrel temperature, the moisture content of the blend, etc. affect physical, structural and sensorial properties of the extruded samples (Mkandawire et al., 2014; Oliveira et al., 2017; Potter et al., 2013). Instead in 3D printing, the printability of the food material strongly depends on the printing conditions, such as nozzle size, feed rate also known as print speed, flow, etc. As 3D printing studies in food sector were developed in the recent years, only few data are available on the effects of the process parameters on the quality of 3D edible objects, and less few information are specifically disposable for cereal-based products.

All of these aside, it is possible to outline some important effects of the principal process variables.

The first factor affecting the quality of the printed materials is the nozzle. In 3D printing, especially in the extrusion-based process, the food material is extruded from a nozzle, so the nozzle diameter is a limiting factor for the food filament. Reviewing some studies, which investigated this parameter, there is evidence to state that decreasing the aperture size of the nozzle, a better object surface and, obviously, a thinner layer thickness are obtained, while with a small diameter nozzle, the force required to extrude samples increased (Kim et al., 2018; Sun et al., 2018).

Print speed, defined as the speed at which the printing happens, was well investigated in some studies. Increased values of print speed in general have a negative impact on surface roughness by breaking the deposited layer or causing deformation, while a positive impact was founded on flatness of the samples (Li et al. 2017, Sun et al., 2018). Height, width and length of printed samples decrease with high print speed values (Li et al., 2017); in this case the high print speed causes a break of the deposited layer and/or a less deposition of the layer amount (Sun et al., 2018). These errors in the deposition of the food material caused the deviations from the projected height, width and length and, in turn, to the deformation of samples. Moreover, Derossi et al. (2018) observed an increase of the pore diameter distributions in 3D samples printed with high print speed. On the contrary, low print speed values bring about a huge discharge and accumulation of food material, thus producing an increment of the weight of the sample and of the layer thickness (Derossi et al., 2018; Sun et al., 2018). In this way, the overall surface quality is scarified.

However, there is a lack of knowledge for a systematic comprehension of the effects of printing variables on the 3D object as well as some other unexplored variables exist for a better optimization of the process.

6.7 Innovative food formulation in 3D printing: the case of cereal-based products enriched with edible insects

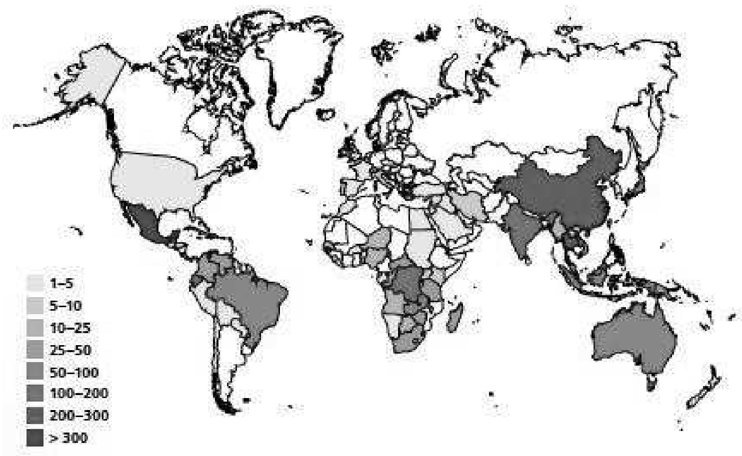
3D printing technology is highly used in food sector to develop personalized products in terms of shape, dimension, color, sensorial preferences of consumers, but also for nutritionally personalized foods; so, current efforts in this technology are focused on the improving the exterior quality of foods as well as on the consumer's health (Lipton, 2017).

From a nutritional point of view, some innovative ingredients such as edible insects could be interesting in achieving high nutritious and functional food. In fact, this is one promising area in enriching cereal-based products, because of their particular nutritional properties, well described in the next paragraph. In addition, in a society where consumers are not accustomed to eating whole insects, the processed forms seem to be the best way to make them acceptable. For all these reasons, current research in 3D printing is being attention to new formulations for developing new products with nutritional added value from edible insects.

6.7.1 Nutritional properties of edible insects

Insects are highly nutritious foods which are consumed by more than two billion of people worldwide; in particular they are an important food source for humans in Africa, Asia and Latin America, where lots of species were recorded (Figure 6.11) and where they have been used as traditional and staple foods, especially among indigenous people (Kinyuru et al., 2009; Rumpold & Schluter, 2015).

Figure 6.11 Edible insect species recorded by country (FAO, 2013)



Jongema (2017) counted more than 2000 edible insects species, which differ a lot for nutritional composition (Rumpold & Schluter 2013). Several studies report the nutrient content of different species of edible insects but the results tremendously vary, because of the used methods, the investigated species, the origin of the insects, the type of rearing, the stage of their lifecycle, the kind of insect diet, etc. (Doberman et al., 2017; Kinyuru et al., 2009; Rumpold & Schluter 2013; Rumpold & Schluter, 2015;). In general, edible insects are a good source of energy, protein, fat, vitamins and minerals (Rumpold & Schluter 2013). In Table 6.1 the minimum and the maximum values of Kcal and major macronutrients in edible insects are shown. Energy values vary from 282 to 762 Kcal/100 g dry weight and they mainly depends on the fat content. The principal components of edible insects are proteins, which range between~30% to~75% of the total dry matter (Doberman et al., 2017). For this reason they represent an alternative source of protein in the human diet, especially for the high availability and digestibility compared with casein and/ or soy (Rumpold & Schluter, 2015). Moreover, the amino acid composition of edible insects meets the amino acid requirements of human adult diet according to the World Health Organization (WHO, 2007); in particular they satisfactorily provide the essential amino acids required for human nutrition.

In addition to protein, the second main component of edible insects is fat, with an average content of about 30% on dry basis, even if several studies demonstrated a high variability of fat content (Rumpold & Schluter, 2015; Ramos-Eldorluy, 1997). This is likely due to that there are some types of "high fat insects", such as caterpillar, palm weevil larvae and wasp, with approximately 60-70% of fat content, which could be used as high energy supplements. Regarding the fatty acids profile, edible insects contain more polyunsaturated fatty acids than either poultry or red meat, while they have very law amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for normal cellular functioning (Doberman et al., 2017). As expected, the fatty acid composition was observed to be highly dependent on the insect feeds (Rumpold & Schluter, 2015)

Insects also contain high amounts of minerals (copper, iron, selenium, zinc, magnesium, etc.) and vitamins (such as biotin, riboflavin, pantothenic acid, folic acid), which are of particular interest for women's and child's diet in the developing countries (Bellucco et al., 2015).

Finally, insects also contain a significant amount of fibre (Table 6.1). The most common form of fibre in insects is chitin, a N-acetylglucosamine present in the insect exoskeleton, which is the main component of their body. Chitin is an indigestible fibre, which improves the immune response of people and can act as defence against some parasitic infections and allergic states (Kourimska & Adamkova, 2016).

To the end, although it is required a still extensive research on the nutrient composition of insects, it is undisputed that they represent an alternative and sustainable protein and fat source. So insects are interesting in human feeding and, because of their particular nutritional composition, they may be included in the common diet of EU consumers in the next future.

Table 6.1 Minimum and maximum values of kcal and selected nutrients of edible insects.

Nutrients*	Minimum value	Maximum value
Energy value (Kcal/100 g)	282[b]	762[a]
Fat (g/100g)	7[a]	77[a]
Protein (g/100g)	32.86[c]	77[d]
Fibre (g/100g)	5[b]	27[e]

*on dry weight

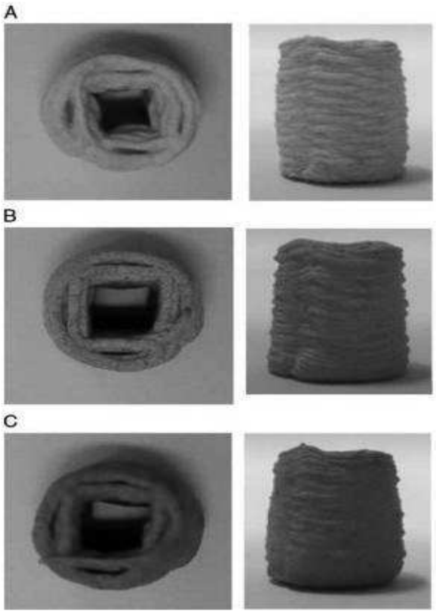
[a] Ramos-Elorduy et al., 1997; [b] Rumpold & Schluter, 2015; [c] Doberman et al., 2017; [d] Ramos-Elorduy et al., 2007; [e] Bednarova, 2013

6.7.1 Current research in 3D printing for obtaining insect-enriched products.

In the Western countries edible insects are gaining increasing interest because of some factors, such as their high protein content and the low environmental costs of production. Despite these benefits, the consumer acceptance in the Western countries has to be improved; to date cultural and psychological barriers need to be overthrown yet. Because of the aversion of people and as reported by some studies, enriching familiar products with edible processed insects in a way they are invisible could be a useful strategy to enhance and boost the use of insect-based products (Le Goff & Delarue, 2017; Hartmann & Siegrist, 2016).

Due to the new interest in the use of edible insects for human nutrition, some researchers are paying close attention to the development of innovative products enriched with edible insects with the use of 3D printing. Severini et al. (2016) produced innovative wheat-based snacks enriched with insect powder (*Tenebrio molitor*) in order to improve the nutritional quality of traditional snacks. Specifically they enhanced the protein content and the amino acid profile of snacks obtained with the addition of insect powder to wheat flour. On the other hand, the substitution of ground insects up to 20 g/100 g d.w. decreased the dough stability, leading to a higher dough deposition during printing, that slightly increased the thickness of the printed dough filament (Derossi et al., 2018). In addition, and as well visible in Figure 6.12, the high dough deposition caused the increase of the diameter of the printed snack and the decrease of their height.

Figure 6.12 Images of 3D printed wheat-based snacks with addition of 0% of T. Molitor powder (A), 10% of T. Molitor powder (B) and 20% of T. Molitor powder (C) (in Severini et al., 2016)



“Insect Au Gratin” is a big project, which looks for new ways of consuming insects by combining entomophagy and the emerging 3D printing technology (Soares, 2018). The designer Susana Soares, in collaboration with the London South Bank University and the University of West England, handled the aesthetics and the development of some prototypes of ready-to-eat and ready-to-cook 3D printed insect dough (Figure 6.13). The dough was previously prepared by mixing insect flour with icing butter, cream cheese, water and gelling agent in order to reach the right consistency to go through the nozzle and to don't collapse.

Figure 6.13 Prototypes of 3D printed insect foods (Soares, 2018)

Also at the Wageningen University researchers are working on edible insect products with 3D printing. They pre-processed Yellow mealworms by drying and milling the larvae in order to obtain a powder, which can be mixed with traditional cereal flours for obtaining 3D printed products (Azzollini & Fogliano, 2018). Another way for using insect powder is to fractionate the different components of the materials, such as soluble and insoluble proteins, lipids, fibre, which enable to obtain foods with different characteristics starting from the same materials. For example is possible to obtain new shapes of foods supported by protein cross-linking, multilayer food system or jelly by state transition of gelling proteins (Derossi et al., 2018).
The use of 3D printing for obtaining edible insect-based products can increase the acceptability of the consumers by developing captivating shapes. Several studies reported that generally consumers reject the idea to eat product with insects, but presenting insects invisibly within familiar preparations, can reduce negative perceptions and increase their acceptance (Le Goff & Delarue, 2017; Hartmann & Siegrist, 2016). These reports suggest that creating appealing products in terms of shape, texture and design with invisible insects, is a good strategy to enhance the consumption of edible insects in Western countries.

6.8 Conclusions

Most of food manufacturing techniques are developed for mass customization, while food designing, in terms of new shapes, structures, flavours and nutritional composition are usually sacrificed. 3D printing is a technology which enables food industry and consumers to obtain personalised foods in all the above-mentioned attributes.

Up to now, some research studies on the 3D food printing were carried out, but new studies have to be conducted in order to achieve a better understanding of this process.

In particular, some aspects need be considered:

1. ingredients and their nutritional compounds have a strong impacts on the rheological properties of a food formulation, which in turn affect the printability of a dough;
2. textural properties are important attributes to determine the sensory quality of a product so by modifying textural characteristics of a food products thanks to the use of 3D printing, it will be well accepted by consumers;
3. some food materials are non printable by nature, thus adding some thickening agents the ideal printability of a food formulation could be obtained;
4. the comprehension of the printing variables are essential for the optimization of the technology.

Last but not least some studies exist on 3D printed products with cereals and insects, too. Most of them gave scarce and still primitive information, so for future perspective we will attend more knowledge in this field.

References

Agerawi,W., Verbonen, P., Vancauwenberghe, V., Bongaers, E., van der Eijnden, E., van Bommel, K., Diaz, J., & Nicolai, B. (2015). Structural-mechanical analysis of cookies produced by conventional and 3D printing techniques. In: *Proceedings of the 29th EFFoST International*, Athens, Greek.

ASTM International (2015), ISO/ASTM52900-15. Standard Terminology for Additive Manufacturing- General Principles- Terminology. ASTM. West Conshohocken, PA.

Azzollini, D., & Fogliano F. *Potential and challenges of edible insects in 3D food printing.* https://3dfoodprintingconference.com/wp-content/uploads/2017/07/Domenico-Azzollini-wur.pdf Accessed 10/01/2018

Balletti, C., Ballarin, M., & Guerra, F. (2017). 3D printing: State of the art and future perspectives. *Journal of Cultural Heritage, 26*(Supplement C), 172-182. doi: https://doi.org/10.1016/j.culher.2017.02.010

Batchelder, J. (1995). *United States Patent No. US5653925 A*

Bellucco, S., Losasso, C., Maggioletti, M., Alonzi, C., Ricci, A., & Paoletti, G. Edible insects: a food security solution or a food safety concern?. Animal frontiers, 5(2), 25-30. doi: 10.2527/af.2015-0016.

Berman, B. (2012). 3-D printing: The new industrial revolution. *Business Horizons, 55*(2), 155-162. doi: https://doi.org/10.1016/j.bushor.2011.11.003

Bednářová, M. (2013). Possibilities of Using Insects as Food in the Czech Republic. Dissertation thesis Mendel University, Brno, 50–92.

Cohen, D.L., Lipton, J.I., Cutler, M., Coulter, D., Vesco, A., & Lipson, H. (2009). Hydrocolloid printing: a novel platform for customized food production. In: *Proceedings of the 21st Solid Freeform Fabrication Symposium*, Austin, USA.

Crump, S.S. (1991). Fast, Precise, safe prototypes with FDM, American Society of Mechanical Engineers. Production Engineering Division, 53-60.

Derossi, A., Caporizzi, R., Azzollini, D., & Severini, C. (2018). Application of 3D printing for customized food. A case on the development of a fruit-based snack for children. *Journal of Food Engineering, 220*(Supplement C), 65-75. doi: https://doi.org/10.1016/j.jfoodeng.2017.05.015

Diaz, J.V., Van Bommel, K.J.C., Noort, M.W., Henket, J., & Brier, P. (2014). Preparing Edible Product, Preferably Food Product Including Bakery Product, and Confectionary Product, Involves Providing Edible Powder Composition, and Subjecting Composition to Selective Laser Sintering. Nederlandse Org Toegepast Natuurwetensch (Nede-C).

Doberman, D., Swift, J.A., & Field, L.M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin, 42*, 293-308. doi: 10.1111/nbu.12291.

FAO. (2013). *Edible insects. Future prospects for food and feed security.* Food and Agriculture Organization of the United Nations (Vol. 171).

Gracia-Julia, A., Hurtado-Pnol, S., Leung, A., & Capellas, M. (2015). Extrusion behavior of food materials in a 3D Food Printer. Pectin based bio-ink formulations for 3-D printing of porous foods. In: P*roceedings of the 29th EFFoSt International*, Athens, Greek.

Godoi, F. C., Prakash, S., & Bhandari, B. R. (2016). 3d printing technologies applied for food design: Status and prospects. *Journal of Food Engineering, 179*, 44-54. doi: 10.1016/j.jfoodeng.2016.01.025

Hamilton, C. A., Alici, G., & in het Panhuis, M. (2018). 3D printing Vegemite and Marmite: Redefining "breadboards". *Journal of Food Engineering, 220*(Supplement C), 83-88. doi: https://doi.org/10.1016/j.jfoodeng.2017.01.008

Hao, L., Mellor, S., Seaman, O., Henderson, J., Sewell, N., & Sloan, M. (2010). Material characterisation and process development for chocolate additive layer manufacturing. *Virtual and Physical Prototyping, 5*(2), 57-64. doi: 10.1080/17452751003753212

Hartmann, C., & Siegrist, M. (2016). Becoming an insectivore: Results of an experiment. *Food Quality and Preference, 51*, 118–122. http://doi.org/10.1016/j.foodqual.2016.03.003

Holland, S., Foster, T., MacNaughtan, W., & Tuck, C. (2018). Design and characterisation of food grade powders and inks for microstructure control using 3D printing. *Journal of Food Engineering, 220*(Supplement C), 12-19. doi: https://doi.org/10.1016/j.jfoodeng.2017.06.008

Jongema, Y. (2017). Worldwide list of recorded edible insects. https://www.wur.nl/upload_mm/8/a/6/0fdfc700-3929-4a74-8b69-f02fd35a1696_Worldwide%20list%20of%20edible%20insects%202017.pdf Accessed 17/12/2017

Kim, H. W., Bae, H., & Park, H. J. (2018). Reprint of: Classification of the printability of selected food for 3D printing: Development of an assessment method using hydrocolloids as reference material. *Journal of Food Engineering, 220*(Supplement C), 28-37. doi: https://doi.org/10.1016/j.jfoodeng.2017.10.023

Kinyuru, J.N., Kenji, G.M., & Njoroge, M.S. (2009). Process development, nutrition and sensory qualities of wheat buns enriched with edible termites (*Macrotermes subhylanus*) from lake Victoria region, Kenya. *African Journal of Food Agriculture, Nutrition and Development, 9*(8), 1739-1750.

Kourimska, L., & Adamkova, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal, 4*, 22-26. doi: http://dx.doi.org/10.1016/j.nfs.2016.07.001.

Le Goff, G., & Delarue, J. (2017). Non-verbal evaluation of acceptance of insect-based products using a simple and holistic analysis of facial expressions. *Food Quality and Preference, 56*, 285-293. http://doi.org/10.1016/j.foodqual.2016.01.008

Le Tohic, C., O'Sullivan, J. J., Drapala, K. P., Chartrin, V., Chan, T., Morrison, A. P., . . . Kelly, A. L. (2018). Effect of 3D printing on the structure and textural properties of processed cheese. *Journal of Food Engineering, 220*(Supplement C), 56-64. doi: https://doi.org/10.1016/j.jfoodeng.2017.02.003

Li, Y., Hu, Y., Cong, W., Zhi, L., & Guo, Z. (2017). Additive manufacturing of aluminia using laser engineered net shaping: effects of deposition variables. *Ceramics International, 43,* 7768-7775. doi: https://dx.doi. org/10.1016/j.ceramint.2017.03.085.

Lille, M., Nurmela, A., Nordlund, E., Metsä-Kortelainen, S., & Sozer, N. (2018). Applicability of protein and fiber-rich food materials in extrusion-based 3D printing. *Journal of Food Engineering, 220*(Supplement C), 20-27. doi: https://doi.org/10.1016/j.jfoodeng.2017.04.034

Lipton, J. I. (2017). Printable food: the technology and its application in human health. *Current Opinion in Biotechnology, 44*, 198-201. doi: https://doi.org/10.1016/j.copbio.2016.11.015

Lipton, J. I., Cutler, M., Nigl, F., Cohen, D., & Lipson, H. (2015). Additive manufacturing for the food industry. *Trends in Food Science and Technology, 43*(1), 114-123. doi: 10.1016/j.tifs.2015.02.004

Lipton, J.I., & Lipson, H. (2011). *United States Patent No. US20120241993 A1*

Lipton, J., Arnold, D., Nigl, F., Lopez, N., Choen, D., Noren, N., Lipson, H. (2010). Multi-material food printing with complex internal structures suitable for conventional post-processing. In: *Proceedings of the 21st Solid Freeform Fabrication Symposium*, Austin, USA.

Liu, Z., Zhang, M., Bhandari, B., & Yang, C. (2018). Impact of rheological properties of mashed potatoes on 3D printing. *Journal of Food Engineering, 220*(Supplement C), 76-82. doi: https://doi.org/10.1016/j.jfoodeng.2017.04.017

Mayor, L., & Sereno, A.M. (2004). Modelling shrinkage during convective drying of food materials: a review. *Journal of Food Engineering, 61* (3), 373-386.

Mkandawire, N. L., Weier, S. A., Weller, C. L., Jackson, D. S., & Rose, D. J. (2015). Composition, in vitro digestibility, and sensory evaluation of extruded whole grain sorghum breakfast cereals. *LWT - Food Science and Technology, 62*(1), 662-667. doi: http://dx.doi.org/10.1016/j.lwt.2014.12.051

McKenna, B.M. (2003). *Texture in Food.* (1st ed.) Woodhead Publishing. Cambridge, England.

Oliveira, L. C., Schmiele, M., & Steel, C. J. (2017). Development of whole grain wheat flour extruded cereal and process impacts on color, expansion, and dry and bowl-life texture. *LWT - Food Science and Technology, 75*, 261-270. doi: http://dx.doi.org/10.1016/j.lwt.2016.08.064

Periard, D., Schaal, N., Schaal, M., Malone, E., & Lipson, H. (2007). Printing food. In: *Proceedings of the 18th Solid Freeform Fabrication Symposium,* Austin, USA.

Potter, R., Stojceska, V., & Plunkett, A. (2013). The use of fruit powders in extruded snacks suitable for Children's diets. *LWT - Food Science and Technology, 51*(2), 537-544. doi: http://dx.doi.org/10.1016/j.lwt.2012.11.015

Ramos-Elorduy, J., Pino, J.M., Prado, E.E., Perez, M.A., Otero, J.L., & de Guevara, O.L. (1997). Nutritional value of edible insects from the state of oaxaca, mexico. *Journal of Food Composition and Analysis, 10*, 142–157.

Ramos-Elorduy, J., Costa Neto, E.M., Pino, J.M., Correa, M.S.C., Garcia-Figueroa, J., & Zetina, D.H. (2007) Knowledge about useful entomofauna in the county of La Purisima Palmar de Bravo, Puebla State, Mexico. *Biotemas, 20*, 121–134.

Ratti, C. (2001). Hot-air and freeze-drying of high value foods: a review. *Journal of Food Engineering, 49* (4), 311-319.

Rumpold, B.A., & Schluter, O. (2015). Insect-based protein sources and their potential for human consumption: nutritional composition and processing. *Animal Frontiers, 5*(2), 20-24. doi: 10.2527/af.2015-0015

Rumpold, B.A., & Schluter, O. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies, 17*, 1-11. doi: http://dx.doi.org/10.1016/j.ifset.2012.11.005.

Severini, C., Derossi, A., & Azzollini, D. (2016). Variables affecting the printability of foods: Preliminary tests on cereal-based products. *Innovative Food Science & Emerging Technologies, 38*, 281-291. doi: https://doi.org/10.1016/j.ifset.2016.10.001

Severini, C., Derossi, A., Ricci, I., Caporizzi, R., & Fiore, A. (2018). Printing a blend of fruit and vegetables. New advances on critical variables and shelf life of 3D edible objects. *Journal of Food Engineering, 220*, 89-100. doi: https://doi.org/10.1016/j.jfoodeng.2017.08.025

Soares, S. Insect au gratin. http://www.susanasoares.com/index.php?id=82 Accessed 03/01/2018

Sun, J., Peng, Z., Zhou, W., Fuh, J. Y. H., Hong, G. S., & Chiu, A. (2015). A Review on 3D Printing for Customized Food Fabrication. *Procedia Manufacturing, 1*(Supplement C), 308-319. doi: https://doi.org/10.1016/j.promfg.2015.09.057

Sun, J., Zhou, W., Yan, L., Huang, D., & Lin, L.-y. (2018). Extrusion-based food printing for digitalized food design and nutrition control. *Journal of Food Engineering, 220*(Supplement C), 1-11. doi: https://doi.org/10.1016/j.jfoodeng.2017.02.028

van der Linden, D. (2015). 3D food printing. Creating shapes and textures. https://www.tno.nl/media/5517/3d_food_printing_march_2015.pdf. Accessed on 15/12/2017

Vancauwenberghe, V., Mbong, V.B.M, Kokalj, T., Wang, Z., Verboven, P., Lammertyn, P., & Nicolaï, B. (2015). Pectin based bio-ink formulations for 3-D printing of porous foods. In: *Proceedings of the 29th EFFoST International*, Athens, Greek.

WHO/FAO/UNU Expert Consultation. (2007). *Protein and amino acid requirements in human nutrition*. World Health Organization technical report series. doi: http://doi.org/ISBN 92 4 120935 6

CHAPTER 7. Development of personalised teff-based snack for women with the use of 3D printing.

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Abstract
3D-printing is an emerging technology, which has been recently used in the food sector for the development of "personalized food", that means food with tailored shape, nutritional and sensorial characteristics adapted for the single person or for a specific category of consumers. The main aim of this study was to obtain three innovative products by 3D printing technology, which meet the micronutrient requirements of women, in particular iron in adolescent, folate in women of childbearing age, calcium in adulthood and also can be considered as 'source of fibre', basing on the EU Regulation 1924/2006. Cocoa powder was chosen for its high content of iron, oranges for their folate content and almonds for their calcium content. These ingredients were used for three food formula, which were then nutritionally balanced, 3D printed and finally backed as a traditional biscuit. All these three personalised food formula satisfied the women's requirements of iron, folate and calcium; in addition, they had high fibre content, so they could obtain the claim basing on the Regulation 1924. Moreover the best operating conditions in 3D printing were identified in order to obtain objects with desired shape and dimension. The snacks were well appreciated by the investigated panel.

Keywords: personalized foods, teff, 3D printing, fiber, micronutrient requirements, women

7.1 Introduction

3D printing is an emerging technology, which recently is widely being applied in several sectors, such as engineering, pharmaceutical, medical, etc. This technology was invented to build 3D object through the deposition of materials layer-by-layer (Godoi et al., 2016). In the last years the growing interest of food science in printing technology has enabled to develop end-products with shape which could not be achievable with traditional techniques. In addition it can satisfy the consumers' demand of 'personalized foods', which are foods specifically designed for a single person or for a certain groups of consumers. These categories of food fulfil simultaneously sensorial preferences and nutritional requirements of people. Such personalized foods involve the idea of a 'personalised nutrition', which has achieving resounding success in recent years because of the increasing interest of people on their health. It is defined as *'the delivery of personalized diets based on information related to people's existing diet and lifestyle and/or phenotypic information (e.g. sex, age, BMI, etc.), health status, and/or genetic data'* (Celis-Morales et al., 2015).

In particular women in their lifetime have specific requirements of certain micronutrients. Particularly iron during adolescence, folate in women of childbearing age, calcium in elderly.

During girlhood, iron needs are elevated as a result of intensive growth and muscular development, and because of considerable losses during period (Mesias et al., 2013). Accordingly to the recommended energy and nutrient intake for the Italian population (LARN), female teenagers needs are of about 18 mg per day (SINU, 2014).

Folate, or vitamin B9, is present in high concentrations in legumes, leafy green vegetables, and some fruits and play a key role in one carbon metabolic reactions that are involved in DNA synthesis and repair, methylation of DNA, cell proliferation, and amino acid synthesis. Folate deficiency is associated with impairments in reproductive health and foetal development, most notably by an increased prevalence in neural tube defects. The latter are severe birth defects of the central nervous system that originate during embryonic development, including spina bifida and anencephaly (Naderi & House, 2018). For these reasons adequate intakes of folate in women of childbearing age is a key factor in protecting foetus against such genetic diseases.

Finally high calcium intake can contribute to the reduced rate of bone loss and fracture incidence in elders (Pu et al., 2016). Despite such recognised functions of calcium, most people still do not reach the recommended amount, specially women in adulthood whose requirements are generally high (Rossini et al., 2002).

In this study teff flour was chosen because of its excellent nutrient profile: it presents high fibre, iron and calcium content, as well as its amino acid composition is well-balanced (Gebremariam et al., 2014). Teff grain is impossible to decorticate, because of the particular dimension if its kernel, so it is consumed as a whole grain and its healthy and functional properties are well documented (Zhu, 2018).

The main aim of this study was to obtain three innovative snack, which meet the micronutrient requirements of women, with the 3D printing technology. Moreover fibre content of 3D printed samples needs to satisfy the minimum amount pinpointed by the EU Regulation 1924/2006, in order to obtain the nutritional claim 'source of fibre'. In addition, consumers might appreciate sensory properties of such products.

7.2 Materials and methods

USDA Food Composition database (release 28) was investigated in order to choose the ingredients with high content of iron, folate and calcium. The selected ingredients were purchased in a local grocery store and used to develop three different snacks. The overall composition of each food formula was reported in Table 7.1.

All ingredients were weighted and mixed in a planetary kneader Cooking Chef KM 086 (JVC-KENWOOD s.p.a., Milano, Italia) for 13 minutes and the dough obtained was used to produce 3D printed snack with a 3D printing machine mod. Delta 2040 (Wasp project, Italy) equipped with the Clay extruder kit 2.00 (Wasp project, Italy). The three-dimensional structure used for food printing was previously designed by using the browser Tinkercad (Autodesk, Inc.). From Tinkercad, a stereolithography interface format file, .stl, was acquired and used as a model to change printing parameters in order to obtain the desired object. CURA 15.05.01 (Ultimaker B.V., The Netherlands) software was used to modify the setting of .stl file. Preliminary tests were conducted in order to identify the best setting parameters, which were reported in Table 7.2. Some other parameters were kept constant throughout all samples, i.e. noozle size was maintained at 1.2 mm, shell thickness at 1.2 mm, layer height at 1.2 mm, travel speed at 12 mm/s. After the printing step, 3D objects were cooked in a conventional oven (mod. Vitality CE116KT - SAMSUNG, South Korea) at 125 °C for 5 min.

Table 7.1 Overall composition of the three food formulas.

Ingredients (%)	Food formula n° 1	Food formula n° 2	Food formula n° 3
Wheat flour	32.09	29.13	16.39
Teff flour	10.7	2.91	8.20
Butter	19.25	17.47	0
Sugar	19.25	17.47	16.39
Egg	12.83	17.47	9.84
Yogurt	0	0	16.39
Cocoa powder	**5.88**	0	0
Orange peel	0	**7.77**	0
Almond flour	0	7.76	**32.79**

Table 7.2 Printing variables

Printing variables	Food formula n° 1	Food formula n° 2	Food formula n° 3
Flow rate (%)	184	211	150
Print speed (mm/s)	64	36	65

On both printed raw and cooked samples a X-ray computed microtomography analysis were conducted with a microCT SkyScan 1174 (Brüker, Belgium). Then the fibre content of cooked samples was estimated with an official method AOAC 32-07.01 (AACC, 2012). Finally, untrained panelists (n=15) for each group of consumers, evaluated the 3D printed samples for overall appearance, colour, odour, crispness, taste, flavour. Panelists were asked to use a scale from 1 (extremely dislike) to 9 (extremely like) for all sensorial attributes.

7.3. Results and discussion

Cocoa powder was selected to meet the iron requirements, orange peel was chosen for its folate content and almond flour because of its high calcium content. These ingredients were used to increase the micronutrient contents of the three food formula.

The main nutritional properties of the printable food formula are reported in Table 7.3. The estimated iron, folate and calcium content are based on the official food composition database USDA (release 28). The Regulation 1324/2006 and the Commission Directive n° 100/2008 stated that a food could be labelled as 'source of....(added minerals and/or vitamins)' if it contains at least 15% of the Recommended Daily Allowance (RDA) of the specific micronutrient. While a food product could be considered as 'a high content of ..... (added minerals and/or vitamins)' if its specific mineral or vitamin content reach the 30% of the RDA for such micronutrient. Basing on these consideration and by comparing the data of Table 3 with the RDA of the selected micronutrient, it can be asserted that a portion of 100 g. of the three 3D printed snack could be labelled as products with 'high iron/folate/and calcium content', for food formula n° 1, 2, and 3, respectively.

Regarding the fibre content, values of 4.521g/100 g d.w., 6.794g/100g d.w., 8.539g/100g d.w for snack with cocoa powder, orange peel and almond flour, respectively, were highlighted (Table 7.3). On the basis of such findings and on the EU Regulation 1924/2006, snack n° 1 could obtain the nutritional claim 'source of fibre', because its fibre content is higher than 3 g/100 g, while snack n° 2 and 3 could be recognized as products with 'high fibre content', due to their values, which are higher than 6 g/ 100 g. It is well known that high fibre intakes provide many health benefits. Studies reveal that high fibre diets could positively affect some intestinal function, glycaemia, cholesterol, as well as it could prevent some chronic diseases (Gebremariam et al., 2014; Vitaglione et al., 2008).

Basing on our results, 3D printed snack could meet the specific micronutrient requirements in the women lifetime, and simultaneously contribute to the improvement of their health condition, because of the higher fibre content.

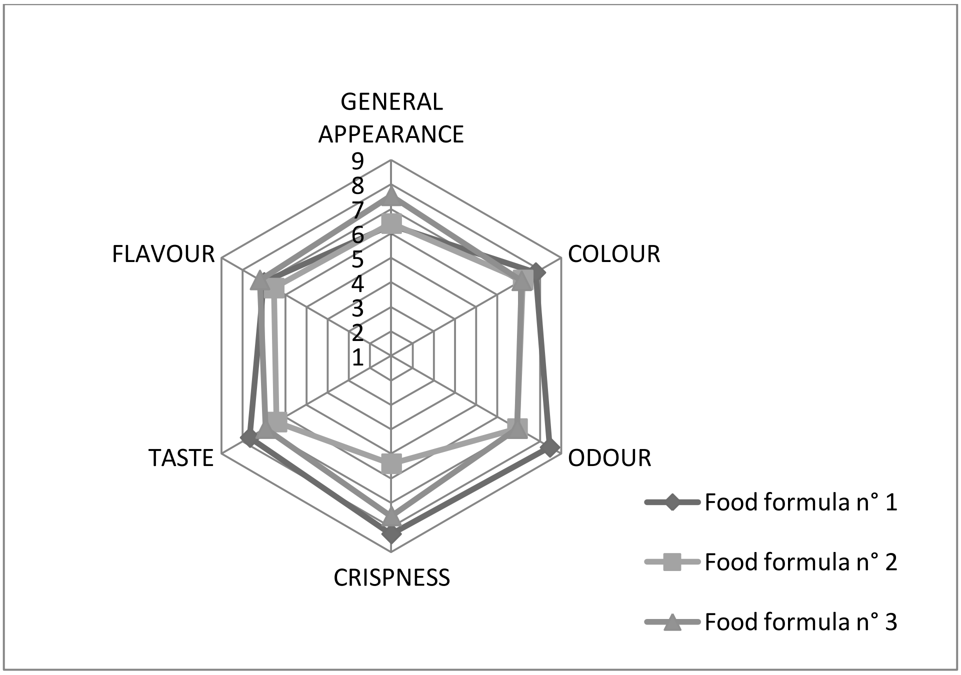
Table 7.3 Iron, folate, calcium and fibre content of the three food formulas

Nutritional content	Food formula n° 1	Food formula n° 2	Food formula n° 3
Iron (mg/100 g)	2.231	-	-
Folate (μg/100 g)	-	29.462	-
Calcium (mg/100 g)	-	-	130.754
Fibre (g/100 g)	4.521	6.794	8.539

Results of the microstructural analysis demonstrated that dimension of printed object well fitted into designed ones, while total porosity significantly changed between samples. Precisely, snack with added cocoa powder showed a porosity of about 64.98%, while food formula with orange peel and almond flour exhibited a porosity of 58.66 and 54.96%, respectively. Such discrepancies were probably a result of the various ingredients added to the food formula, which could differently affect the printing process. Furthermore the total porosity of printed samples was also investigated after the cooking process, and, as a result, it increased. It is well known that during a cooking process vapour production occurs, which could lead to the creation of new pores as well as to the growth of the existing ones. Similar results were highlighted by Severini et al. (2016), who produced 3D printed wheat-based snack.

The sensory evaluation of the 3D printed snack, shown in Figure 7.1, reveals that snack with cacao powder were overall more appreciated. Specifically the targeted group of consumers of such snack, which were adolescent, well marked all the attributed investigated, except of *flavour* and general appearance which were high in snack obtained with almond flour. This latter result was expected because almond-based food formula well flowed through the nozzle. It is well known as the printability, accuracy and replication during 3D printing process are highly governed by the rheological properties of the material (Derossi et al., 2018; Lille et al., 2018; Godoi et al., 2016). Probably the peculiar viscosity of the dough, which was affected by the ingredients used, lead to end-products whose shape was consistent with the design. As a result, these snack were highly appreciated by the selected group of consumer, which were, in this case, elderly women.

Figure 7.1 Sensorial evaluation of 3D printed snack



7.4 Conclusion

This study proved that 3D printing technology was a suitable process to obtain personalized foods. 3D printed snack met the micronutrient requirements of women, particularly iron in food formula with cocoa powder, folate due to orange peel and calcium in almond-based snack. These minerals/vitamins reached the RDA percentage requested to obtain the nutritional claim for such micronutrient. Because of the higher fibre content and its recognized role in protecting human body from some diseases, 3D snack also contribute to the women' health condition.

Undoubtedly 3D printed snack were well appreciated by the panelists, assuming that consumers could accept this innovative and personalized products. Moreover, 3D printing technology presents high degree of freedom; particularly, it allows obtaining complex and identical shape. So further improvements seem necessary, specifically on the general appearance of snack.

References

AACC (2012). *American Association of Cereal Chemists, Approved Methods*, 11th ed. Method 32-07.01 St. Paul, MN.

Celis-Morales, C., Livingstone, K.M., Marsaux, C.F., …., et al. (2015). Design and baseline characteristics of the Food4me study: a web-based randomised controlled trial of personalised nutrition in seven European countries. *Genes and Nutrition*, 10:450.

Derossi A., Caporizzi R., Azzolini D., Severini C. (2018). Application of 3D printing for customized food. A case on the development of a fruit-based snack for children. *Journal of Food Engineering, 220*, 65-75. DOI: 10.1016/j.jfoodeng.2017.05.015

Gebremariam, M. M., Zarnkow, M., & Becker, T. (2014). Teff (Eragrostis tef) as a raw material for malting, brewing and manufacturing of gluten-free foods and beverages: a review. *Journal of Food Science and Technology, 51*(11), 2881-2895.

Godoi, F. C., Prakash, S., & Bhandari, B. R. (2016). 3d printing technologies applied for food design: Status and prospects. *Journal of Food Engineering, 179*: 44-54.

Lille, M., Nurmela, A., Nordlund, E., Metsä-Kortelainen, S., & Sozer, N. (2018). Applicability of protein and fiber-rich food materials in extrusion-based 3D printing. *Journal of Food Engineering, 220*(Supplement C): 20-27.

Mesias, M., Seiquer, I., Navarro, M.P. (2013). Iron nutrition in adolescence. *Critical Review in Food Science and Nutrition,* 53(11): 1226-1237.

Nader, N., House, J.D. (2018). Recent developments in folate nutrition. *Advances in Food Science and Nutrition*, in press

Pu, F., Chen, N., Xue, S. (2016). Calcium intake, calcium homeostasis and health. *Food Science and Human Wellness*, 5(1): 8-16.

Rossini, M., Adami, S. (2002). Calcium and vitamin D in aging patients: clinical and economical issues. *Trends in Medicine, 2*(1): 9-19

Severini, C., Derossi, A., & Azzollini, D. (2016). Variables affecting the printability of foods: Preliminary tests on cereal-based products. *Innovative Food Science & Emerging Technologies, 38*: 281-291.

Società Italiana di Nutrizione Umana. (2014), *Livelli di Assunzione di Riferimento di Nutrienti ed energia per la popolazione italiana IV revision*. SICS, Italy.

United States Department of Agriculture (USDA) - Supplements. Agricultural research service. National nutrient database for Standard Reference, R. S. v., The National Agricultural Library. Reference Release 2

CHAPTER 8. General conclusions and future implications

8.1 Cultivation of minor cereals in a Mediterranean area

The first aim of this research was to investigate the adaptability of some minor cereal crops in a Mediterranean environment, in particular in the south of Italy, which is generally characterized by scarce rainfall and hot dry summer. So, in this first part of the research, three minor cereals, such as millet, sorghum and teff, were grown under full and limited irrigation and some yield parameters and nutritional aspects were studied.

The major findings of such experiments were that deficit irrigation treatment significantly affected the yield of minor cereals, except for teff. In addition sorghum exhibited the highest free phenolic content, millet showed the highest bound phenolic content but the lowest free phenolic content and total antioxidant capacity. Moreover the highest total antioxidant capacity was detected for teff grains. Thanks to these findings, teff was identified as the most adapted crop in the area of the study, under deficit irrigation.

The efficient use of water in agriculture is of outmost importance for the conservation of the limited resources and this research gave several important information regarding this issue. In a context of sustainable water saving, a useful strategy is the diversification of production and consumption habits. Thus, the use of broader range of plant species, in particular those identified as underutilized and needing low input of water, can partially solve the problem of water scarcity and also contribute to crop biodiversity. Among these emerging crops, minor cereals are attracting more attention because they could contribute to producer's profitability and to consumer's health too, due to their high functional and nutritional properties.

On the other hand a deficit of irrigation could lead to a decrease in yield, so the knowledge of the response of crops to low water management needs to be well understood. In the context of irrigation, the relationship between yield and applied water is of huge economically importance. For future research could be useful to investigate different *cultivar* for each species, because of the multiplicity in their response and different irrigation levels, to well understand what is the best crop management for the best quality of a crop production.

The cultivation of teff under limited irrigation condition could be a first example of crops grown in a friendly environmental, because of the sustainable use of a very scarce resource, which is, in this case, water. Fortunately, consumers are willing to pay for special foods, particularly those associated with environmental friendly farming practices. Thereby, farmers will be convinced about the economic benefits to dedicate more larger areas to the cultivation of even more crops and it should be possible to ensure consumer's satisfaction, strengthening their willingness to pay a reasonably higher price for products with high health benefits and environmental friendly.

These findings could establish the basis for further researches to implement the knowledge about the effect of deficit irrigation on crops cultivated in a Mediterranean area. In particular teff grain could be a suitable crop in such area because of its high adaptability to harsh environmental conditions, specifically in terms of yield, and due to its high antioxidant capacity.

8.2 Extrusion cooking and the potential use of teff flour in ready-to-eat breakfast cereals

Extrusion cooking technology is identified as a useful process in respect of the development of a healthy snack because of the distinct textural as well as nutritional properties of the end products. So, it was used in order to develop extruded breakfast cereals with teff flour, with the main aim to improve the knowledge regarding the use of this new cereal to obtain innovative and healthy end products. In addition the effect of process parameters were investigated. Various properties of extruded samples were analysed through this second part of the thesis, from structural to functional characteristics.

First of all, it was observed that the incorporation of teff flour significantly changed the physical and sensory characteristics of extruded cereals, in particular lightness and crispness. However, process management of extruded cereal breakfast, by modifying process conditions, allows creating desirable quality of final products. For example, temperature of 120°C lead to a less hard and more crispiest products, which are more likely accepted by consumers.

Moreover, varying the process parameters and increasing teff flour addition, microstructural properties of samples change considerably and these characteristics could affect structural properties of extruded products. When the lowest feed moisture was used the majority of

the wall thickness was small in size, according to the highest expansion. Instead, samples obtained at the highest temperature and with the highest teff flour addition showed the greatest number of small pores which led to a low crispness of such samples.
Regarding the antioxidant capacity of extruded samples, the first paper (chapter 4) allow to state that total antioxidant capacity of extruded samples was strictly related with the teff flour addition. In fact the highest total antioxidant capacity was highlighted in extruded samples with 70% of teff flour respect to samples with 30% of teff flour.
In the second paper (chapter 5) dedicated to extruded cereal breakfast obtained with teff flour a deeper understanding of the functional compounds related with the antioxidant capacity was achieved. Results of this second study suggest that teff flour serves as a good source of polyphenol, which are the major contributors to the antioxidant capacity in extruded ready-to-eat breakfast cereals, and dietary fiber. In addition, by controlling some extrusion conditions, such as temperature and feed moisture, the antioxidant capacity of gluten free expanded products could be enhanced.
All these findings suggest that extrusion technology, under optimal conditions, seems promising for the preparation of gluten free products with high functional properties and with good structural characteristics, which could lead to high acceptability of consumers.
By combining results of the effect of teff flour addition and extrusion process parameter on structural and sensorial characteristics of extruded cereal-based breakfast, producers could be able to manufacture such products, which well fit the increasing demand of healthy products, especially for the gluten free market.
In the future, further investigation could be conducted in order to well understand the effect of teff flour addition on some other nutritional compounds. As an example the extent to which carbohydrate is digested and absorbed in the small intestine determines its health effect. The rate of carbohydrate digestion could be characterized by its glycemic index, which depends on the macroscopic structure of the food as well as on the changes occurred during the process. It is known as in vitro starch digestibility of teff was found to be significantly lower than that of wheat. In addition extrusion process could affect starch digestibility by increasing the level of resistant starch, in particular the RS3 portion, also known as retrograded amylose, which resist to the digestion in the small intestine.

Research opportunities exist to improve the status of teff as a popular and healthy food item. In addition, since extruded cereal-based breakfast are consumed daily by the majority of the population, food industry should focus its attention on creating products which naturally contain functional ingredients.

8.3 The use of 3D food printing to obtain personalized foods.

Additive manufacturing, more commonly referred as 3D printing, is currently of significant interest for numerous food application. It offers a wide range of possibilities with respect to customization, precision and innovation for the creation of novel shapes and textures, and a potential for generation of healthy products.

In this research the most important advances on the application of 3D printing for cereal-based products were analyzed. Major conclusions of such review were that ingredients and their nutritional compounds have a strong impact on the rheological properties of food formulation, which in turn affect the printability of dough. In addition, some food materials, such as vegetables, are non printable by nature, thus adding some thickening agents the ideal printability of a food formulation could be obtained. Finally, the comprehension of the printing variables is essential for the optimization of the technology, but knowledge on the effect of these parameters is still primitive and further researches have to be conducted.

Then, 3D printing technology was applied for the development of snacks specifically designed for women. In particular cocoa powder was chosen to increase iron, because of the high needs in adolescent; oranges were selected to augment folate content, which is required in women of childbearing age; finally calcium needs raise in adulthood and it was increased by adding almond flour. Because of its excellent nutritional profile, teff flour was used to enhance the nutritional properties of all the designed snacks. All these three personalised food formulas satisfied the women’s micronutrient requirements; in addition, they had high fibre content, so they could obtain the claim basing on the Regulation 1924/2006. Results of this study proved that 3D printing technology was a suitable process to obtain personalized foods, with desired shape and dimension. Last but not least 3D printed snack were well appreciated by the panelist, assuming that consumers could accept this innovative and personalized products. Concerning microstructural analysis it was demonstrated that total porosity significantly changed among samples as a result of the various ingredients added to the food formula, which differently affected the printing process. This aspect seems to have great importance when the aim is to design a 3D printed food with specific features, for instance of chewiness or crunchiness, which depend on the degree of filling of designed food object. However, the fundamental principles underlying how this process affect food microstructure, and consequently sensory attributes, need to be further investigated to allow for greater diversification in more application areas. In the future the material's strength, viscosity, dimensional stability, resistance to heat and moisture, and colour stability will need a more careful evaluation.

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