

Impact of AM fungi on functional properties and sensorial attributes of three cassava/wheat composite flours and biscuits.

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Abstract

The study aimed to investigate the influence of AM fungi inoculation and the partial substitution of wheat flour with cassava flour on some functional properties of composite flours, and to do analysis of the hedonic properties of biscuits from the most promoted composite flours. Wheat flour was substituted with three cassava flours at the rate of 25, 50 and 75%, followed by the analysis of the functional properties of flours. Cassava flour was from both non-AM and AM fungi plants. The 50/50 flour formula was used to make biscuits for each variety. Results show that composite flour from AM fungi inoculated plants has better functional properties characteristics than wheat flour and flour from non-AM fungi inoculated plants. Cassava variety also impacts functional properties. Contrast was noted with appreciation of biscuits from the 50/50 composite flour. The biscuits made with flour from none AM fungi AE and 693TME cassava varieties show better sensorial scores than none AM fungi, while the contrary was recorded with the 92/0326 cassava variety. This study highlights the emergency to include nutritive criteria while doing plant breeding in order to better fight hunger across the world population. Such study must be extended to other cassava varieties and crops.

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I. Introduction

Wheat base pastry products have considered input in the food systems across the world. Wheat production account between the top five cereals production in the world, with 808 million tons of grain in 2022 (FAO, 2023). Approximately 20 % of food calories and proteins gain by the world population come from wheat (Shiferaw et al., 2013). Key agricultural strategies allow improvement of this crop production within the past year, including breeding programs with several orientations (Reynolds and Braun, 2022). Wheat production is facing a global average rate decrease per year and the high demand of the growing population of the world (Shiferaw et al., 2013; Singh et al., 2022). Thus, modern technologies can contribute both to improve wheat production, and the production of alternative crops, potentially useful in the substitution of wheat flour in the pastry foods process. Potential consequence might be the alleviation of such drastic situation.

Beneficial microbes, such as arbuscular mycorrhizal AM fungi could greatly contribute to solve the problem. AM Fungi are organisms undergoing symbiotic relations with several plants, with the exchange of nutrients involving carbon molecules from host plants to fungi, and minerals, including nitrogen, from AM Fungi to host plants (Liu et al., 2013). The carbon/nitrogen balance in plants may determine its nutritive status and the sensorial quality of outcome and derive foods. Field application as fertilizers worldwide was shown to increase by 5 % and 20 % straw and grain yield. Additionally, they increased the grain N and Zn content by 31 % and 13 %, respectively (Marrassini et al., 2024). More over field inoculation of cassava plant with AM fungi improve tuber and leave yields, improve nutrients content (total carbohydrates, lipids proteins, some minerals) and decrease anti-nutrients content (cyanides, oxalate, phytates, tannins) in tubers and leaves (Mbassi et al., 2019; Mbassi et al., 2024). Processing of cassava tuber into sticks records the improvement of the chemicals and sensorial quality of derived products following AM fungi inoculation (Mbassi et al., 2024). It was found that inoculation with AM Fungi increased grain yield and quality, especially bioactive compounds and protein contents (Jerbi et al., 2022). To date, comprehensive investigations into the impacts of AM fungi inoculation on yield protein composition, gluten secondary structure, dough characteristics, baking quality, and their interrelationships are still not well illustrated.

Cassava (*Manihot esculenta* Crantz) is an important tuber crop cultivated in tropical and subtropical regions of the world with a strong tolerance against soil infertility and water stress (Idris et al., 2020). In Africa and South America, cassava is consumed as an important staple food by more than 800 million people (Obojiofor et al., 2021). Fresh cassava roots are difficult to store and usually have a short shelf life of only 1-2 days after harvest because of their postharvest physiological deterioration (Yan et al., 2022; Ma et al., 2022). Therefore, cassava is generally prepared as flour by peeling, crushing, and drying. The production of cassava flour (CF) can prolong the shelf-life of cassava and further reduce cyanide glucosides content to safe levels (Onyenwoke et al., 2015). Cassava flour has attractive advantages in terms of starch, with low retro-gradation, high water-binding ability, and high fiber and mineral content compared with cereal flours. Numerous studies and applications demonstrated the feasibility of partially replacing wheat flour with cassava flour in cakes (Vega et al., 2018), biscuits (Oliveira et al., 2021), bread (Chisenga et al., 2020), and noodles (Wangtueai et al., 2020). Ingredients to be used in composite flour blends must meet a number of criteria including: availability, acceptability and provide necessary nutritional requirements (Akobundu et al., 1998). People are consistent with the fact that, the food produced with cassava flour provided more carbohydrates and low lipid and protein content (Vega et al., 2018; Okafor et al., 2017). A growing literature showed that the sensory quality of bread with a substitution level of 10% or 20% is generally acceptable. Knowledge is growing on the technology of substitution of wheat flour by the flour from other plants but few researchers questioning about the influence of the growth condition of plant like AM fungi inoculation on the functional properties of composite flour, substitution rate, and the sensorial properties of the outcome products. Thus, the aim of this study was to investigate the influence of AM fungi inoculation and the partial substitution of wheat flour with cassava flour at three levels on the functional properties of composite flours. Analysis of AM fungi on the hedonic properties of biscuits from the most promoted composite flours will be also done.

II. Material and Methods

The experiments were conducted in the Soil Microbiology Laboratory in the Biotechnology Centre at the Yaoundé I University in Cameroon. Raw materials, including wheat flour, rice flour, were procured from the local market. Initial moisture content of flours and biscuits was determined using air oven drying method as recommended by AOAC (2000). Cassava tuber of each variety harvest at maturity and divided into two groups: one growth with AM fungi inoculation and the second without AM fungi were provided by the Soil Microbiology Laboratory of the University of Yaoundé I. They were carried to the Laboratory of Food Technology, the Institute of Agricultural Research for Development, Yaoundé in Cameroon for flour production.

II.1 Productions of cassava flours

The cassava tubers were cleaned, peeled, washed and kept in water to avoid discoloration. The roots were then cut into small chips, drained and dried at 50°C for 8h. When dry, cassava chip was milled using a hammer mill apparatus. Two groups of flour were obtained for each variety including non-AM fungi flour (T) and AM fungi flour (M). The flours were store in polythene bags until used for analysis.

II.2 Composite flours preparation

Composite flour was made by substitution of wheat flour with cassava flour at the rate of 25, 50 and 75%, making 3 formulations for each flour (Table 1). Each composite flour was mixed and stored for analysis.

Table: substitution rate of wheat flours

Proportions (%)					
Cont	100	75	50	25	
AE	0	25	50	75	
AEm	0	25	50	75	
92/0326	0	25	50	75	
92/0326m	0	25	50	75	
693TME	0	25	50	75	
693TME m	0	25	50	75	

Cont=wheat flour; AE, 92/0326 and 693TME are cassava variety; AEm, 92/0326m and 693TME m are cassava variety from AM plants.

II.3 Evaluation of functional properties of composite flours

The functional properties of composite flours include swelling capacity (ml), water absorption capacity (WAC, %), oil absorption capacity (OAC, %), Emulsion activity (EA, %), Gelatinization temperature (GT, °C). The swelling capacity was measured following the method described by Okaka and Potter (1977). Graduated cylinder of 100 mL was filled with flour to 10 mL mark. The distilled water was added to give a total volume of 50 mL. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension

was inverted again after 2 min and left to stand for a further 8 min. The volume occupied by the sample was taken after the 8th min.

The water absorption capacity of the flours was determined using the method of Sosulski et al. (1976). One gram of sample was mixed with 10 mL distilled water and allow to stand at ambient temperature ($30\pm 2^{\circ}\text{C}$) for 30 min, and centrifuged for 30 min at 3,000 rpm. Water absorption was recorded as per cent of water bound per gram flour.

The oil absorption capacity was evaluated using the method of Sosulski et al. (1976). One gram of sample mixed with 10 mL olive oil and allow to stand at ambient temperature ($30\pm 2^{\circ}\text{C}$) for 30 min, then centrifuged for 30 min at 300 rpm. Oil absorption was examined as percent oil bound per gram flour.

The emulsion activity was tested following the method describe by Yasumatsu et al. (1972). One gram of sample was mixed with 10 mL distilled water and 10 mL olive oil in calibrated centrifuge tube. The mixture was centrifuged at $2000 \times g$ for 5 min. The ratio of the height of emulsion layer to the total height of the mixture was calculated as emulsion activity in percentage.

Gelatinization temperature was determined by Shinde (2001). One-gram flour sample was weighed accurately in triplicate and transferred to 20 mL screw capped tubes. Ten mL of water was added to each sample. The samples were heated slowly in a water bath until they formed a solid gel. At complete gel formation, the respective temperature was measured and taken as the gelatinization temperature.

II.4 Production of biscuits.

Production was done according to the method described by the American Association of Cereal Chemists (AACC, 2000) with a few modifications. The ingredients were weighed as follows: 60g of each flour (table 1), 50.0g of fine sugar, 1.0 egg (50.0g), 28.0g of shortening, 0.93g of salt, 1.11g of sodium chloride and 1g of vanilla. The ingredients were properly watered and mixed to form a paste, introduced into the moulds before baking at 180°C for 20 min. After cooking, the biscuits were taken out of the oven, cooled and introduced into the plastic bags, then stored for analysis. A total of 22 groups of biscuits were produced, corresponding to flours. They are: W=Wheat, $\text{AE}_{25,50,75}$ =AE biscuits type at three rates of substitution, $\text{AE}_m 25,50,75$ =AE mycorrhiza biscuits type at three rates of substitution, $92/0326_{25,50,75}$ = 92/0326 biscuits type at three rates of substitution, $92/0326m_{25,50,75}$ = 92/0326 mycorrhiza biscuits type at three rates of substitution, $\text{TME}_{25,50,75}$ =TME biscuits type at three rates of substitution, $693\text{TME}_m 25,50,75$ =693TME_m mycorrhiza biscuits type at three rates of substitution.

II.5 Sensorial evaluations.

A semi-trained panel consisting of both genders, with more than 10 judges from different age groups and varying eating habits, was constituted to evaluate the quality. The panelists were selected from the faculty staff and students of the faculty of Sciences, Department of Biochemistry, and the Biotechnology Center University of Yaoundé I. Samples were served to the panelists and they were asked to rate the acceptability of the product through sense of organs. The overall acceptability of biscuits was rated on the basis of 5- point hedonic scale ranging from 1 (extremely dislike) to 5 (extremely like). Prior to the test, each panelist was give its agreement by signing a consent note. The data obtained from the various experiments were recorded during the study and were subjected to statistical analysis as per method of "Analysis of Variance" by Factorial Randomized Block Design. The significant difference between the means was tested against the critical difference at 5 % level of significance SPSS software was used for analyze the recorded data.

III. Results

III.1 92/0326 varieties

The functional properties of wheat flour at various substitution rates from the 92/0326 cassava variety were studied and recorded (Figure 1). With reference to pure wheat flour, a significant increase was recorded for the SC, WAC, and OAC parameters at all substitution rates except 75/25 T and 25/75 M for SC, 75/25 T for WAC, and 25/75 for OAC. The highest increase ratios were 1.41, 1.13, and 1.72 for SC, WAC, and OAC, respectively. Paradoxically, a significant decrease in EC was observed for all substitution rates of composite flours compared to pure wheat flour. A significant increase in GT was recorded only at the 75/25 M and 25/75 M substitution rates compared to pure wheat flour. The influence of cassava growing conditions on the studied flour parameters was significant. Thus, a significant increase in OAC and GT was observed between composite flours from plants inoculated with AM fungi compared to non-AM fungi at all substitution doses. EC and WAC parameters showed a contrasting effect between flours from AM fungi and non-AM fungi plants, with a reduction in EC at the 50/50 dose and a reduction in WAC at the 25/78 dose. SC increased at the 75/25 dose, remained stable at the 50/50 dose, and decreased at the 25/75 dose in flours from plants inoculated with AM fungi compared to non-inoculated plants.

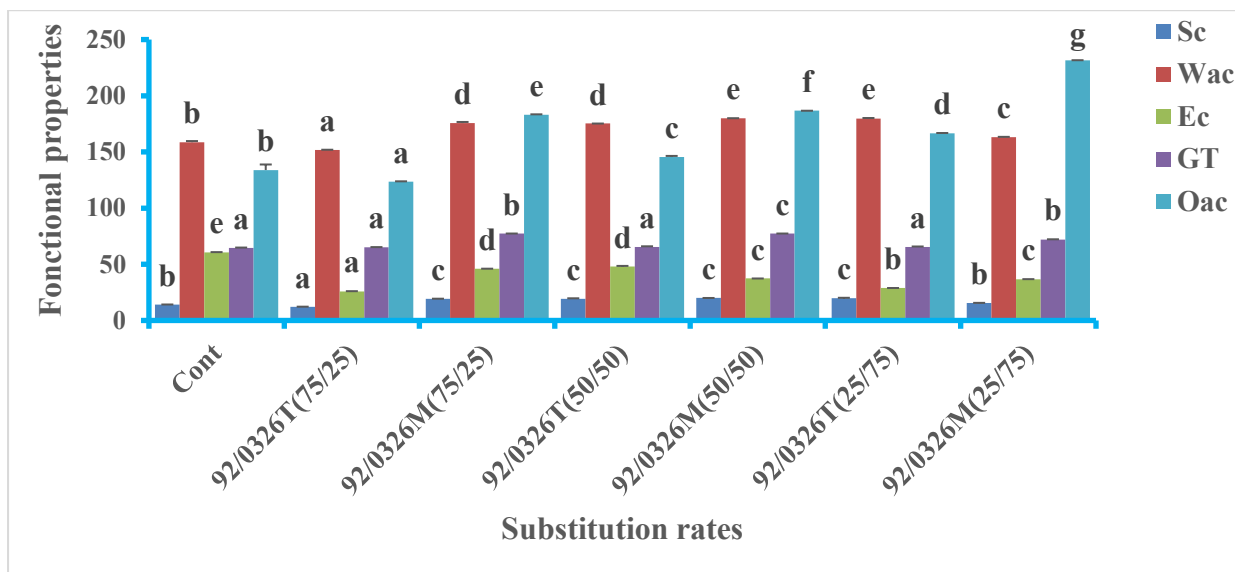


Figure 1: Impact of AM fungi inoculation and substitution rate (wheat/cassava) on some functional properties of flour from 92/0326 cassava varieties.

Cont=wheat flour; 92/0326=cassava variety; (75/25,50/50,25/75) =flour substitution rates of none AM Fungi wheat/cassava; (75/25,50/50,25/75) m =flour substitution rates from AM Fungi wheat/cassava. SC=swelling capacity; WAC=water absorption capacity; OAC=oil absorption capacity; EC=emulsion capacity; GT=gelatinization temperature.

III.2 693TME varieties

The functional properties of flour from the 693TME cassava variety were evaluated (Figure 2). The results show a significant increase in WAC, GT, and OAC at all substitution rates, regardless of the origin of the cassava flour used, compared to pure wheat flour. Furthermore, a significant increase in EC is observed only with substitution rates containing cassava flour from plants inoculated with AM fungi, compared to pure wheat flour. SC, on the other hand, shows a significant increase compared to pure wheat at 50/50 substitution rate, and at the 75/25 dose made with cassava flour from plants inoculated with AM fungi. The formulation of flours with tubers from AM fungi inoculated cassava plants affects the studied parameters in various ways. Thus, EC increases significantly with inoculation at all substitution rates, WAC at the rate of 75/25 and 50/50, GT only at the rate of 50/50, and SC at dose 75/25. OAC increases significantly at dose 50/50 and decreases at dose 25/75.

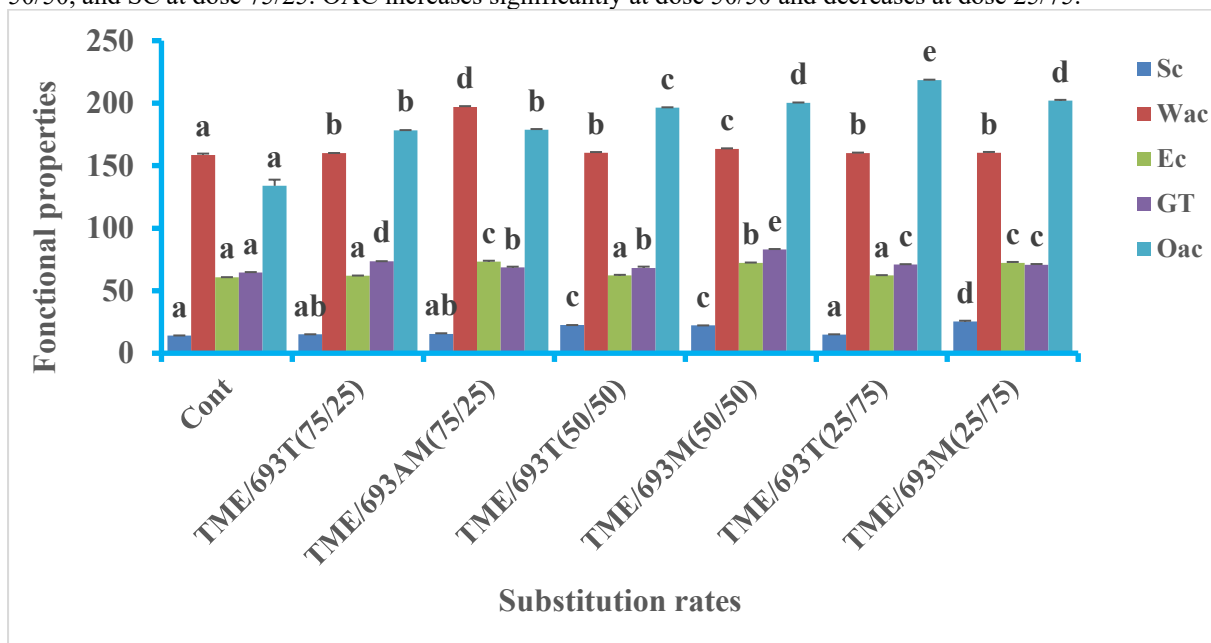


Figure 2: Impact of AM fungi inoculation and substitution rate on some functional properties of flour from 693TME cassava varieties.

Cont=wheat flour; TME/693=cassava variety; (75/25,50/50,25/75) =flour substitution rates of none AM Fungi wheat/cassava; (75/25,50/50,25/75) m =flour substitution rates from AM Fungi wheat/cassava. SC=swelling capacity; WAC=water absorption capacity; OAC=oil absorption capacity; EC=emulsion capacity; GT=gelatinization temperature.

III.3 AE varieties

The functional properties, including SC, WAC, OAC, EC, and GT, of pure wheat flour and composite flour at different substitution rates with cassava flour (variety AE) were evaluated (Figure 3). The results show that OAC increases significantly in composite flours compared to pure wheat flour, regardless of the substitution rate and the growing conditions of the cassava tubers used, while GT shows an inverse trend. EC, on the other hand, shows a significant increase compared to pure wheat flour at all substitution rates when cassava flour was from un-inoculated plants. WAC and SC exhibit highly variable values, with a significant increase in WAC at the (25/75)T, (50/50)M, and (75/25)M rates, as well as in SC at the (75/25)T, 50/50, and (75/25)M rates, compared to pure wheat flour. The impact of cassava plant inoculation with AM fungi on the studied parameters at the different substitution rates shows multiple orientations. Thus, inoculation leads to a significant decrease in EC, OAC and GT at all substitution rates, an increase followed by a significant decrease in WAC at the rates of 75/25, 50/50 and 25/75, respectively, and finally an increase in SC at rates of 50/50 and 25/75.

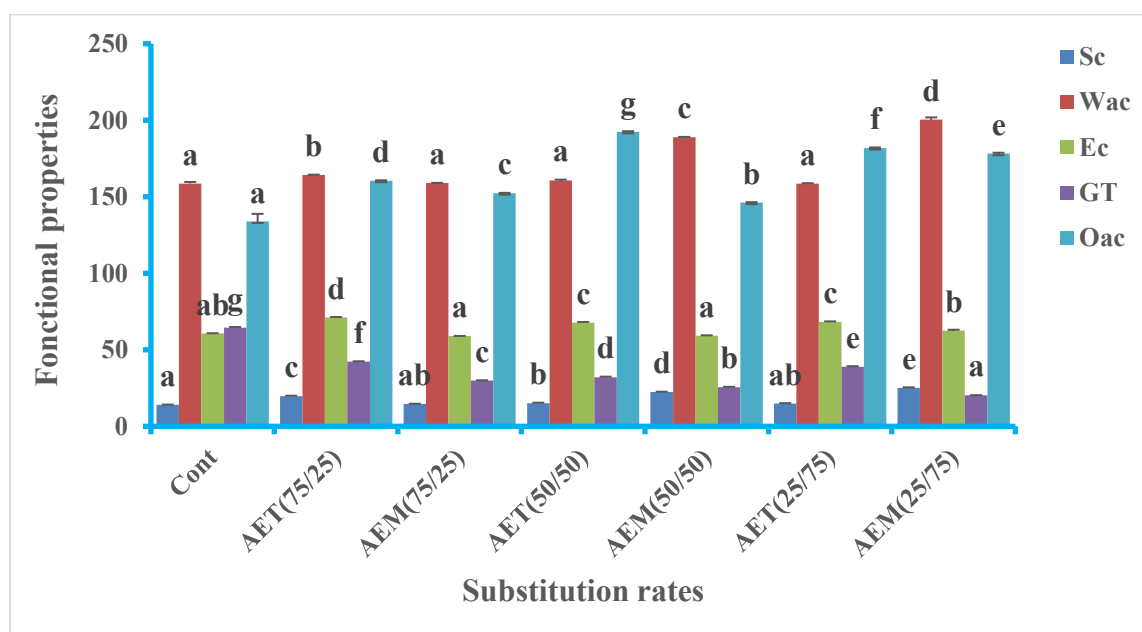


Figure 3: Impact of AM fungi inoculation and substitution rate on some functional properties of flour from AE cassava varieties.

Cont=wheat flour; AE=cassava variety; (75/25,50/50,25/75) =flour substitution rates of none AM Fungi wheat/cassava; (75/25,50/50,25/75)m =flour substitution rates from AM Fungi wheat/cassava. SC=swelling capacity; WAC=water absorption capacity; OAC=oil absorption capacity; EC=emulsion capacity; GT=gelatinization temperature.

A principal component analysis of the functional properties of composite flours across all substitution rates and the growing conditions of the cassava plant varieties used was performed, and the results are presented in Figure 4. This analysis shows that: data are 32.61 % close to the horizontal axis, 28.30 % close to the vertical axis, and 60.91 % close to both axes. The treatments, including cassava varieties and their growing conditions, as well as substitution rates, are grouped into four categories.

The first consists of TME693m (25/75, 50/50, 75/25), TME693t (50/50); 92/0326m (75/25, 50/50) composite flours, which are close to EC and SC.

The second consists of AEm (25/75, 75/25, 50/50,); AEt (75/25); 92/0326t (50/50, 25/75) composite flours which are close to WAC.

The third consists of TME693t (25/75, 75/25); 92/0326t (25/75) composite flours, which are close to GT.

The last group consists of AEt (50/50, 25/75) cont and 92/0326t (75/25) that have no particular correlation with the functional parameters studied.

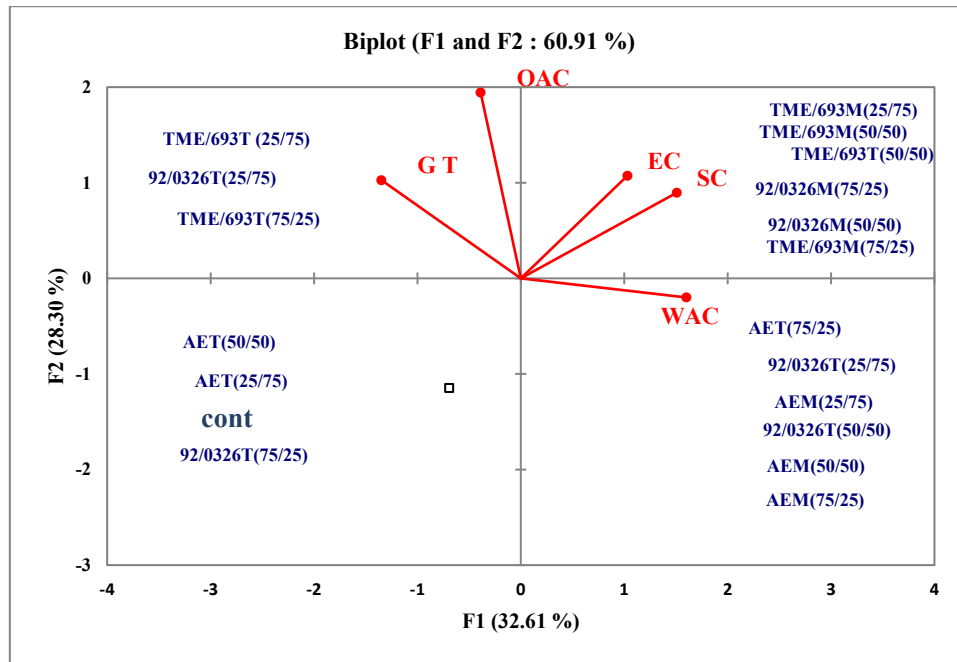


Figure 5: Principal component analysis including cassava varieties, substitution rates and functional properties. AET, 326T, 693TME=none mycorrhizal cassava variety; AEm, 326m, 693TME= mycorrhizal cassava variety. Cont=wheat flour. SC=swelling capacity; WAC=water absorption capacity; OAC=oil absorption capacity; EC=emulsion capacity; GT=gelatinization temperature

III.4 Sensorial analyses

Wheat flour and 50/50 composite (wheat/cassava) flour from AM fungi plants or not were used to make the biscuits, and the sensory properties were tested (Figure 5). Related to the AE cassava variety, biscuits made from composite flour containing cassava from plants inoculated with AM fungi show significantly lower sensory scores than those of composite flour containing cassava from non-inoculated plants. This was observed for all the studied sensorial attributes. Related to the 693TME cassava variety, whether the biscuits are made from composite flour containing cassava from inoculated AM fungi plants or not, no significant differences in scores of any sensory attribute were recorded. Regardless of the origin of the cassava flour used for the composition, the texture attribute has significantly less contribution to the sensory test than the other attributes. The results of the sensory analysis of biscuits made with 0326 cassava variety show highly diverse attribute scores with a real contrast between Am fungi inoculated plant flour producer or not. The color attribute of biscuits made with cassava flour from plants inoculated with AM fungi has a significantly lower score than flour from un-inoculated plants. Inoculation of cassava plants with AM fungi leads to a significant increase in the scores of all other sensory attributes, including general acceptability. The rates of contribution of attributes to the GA of biscuits made with flour from Am fungi uninoculated plants are highly variable, with a predominant contribution of color and a lesser contribution of smell

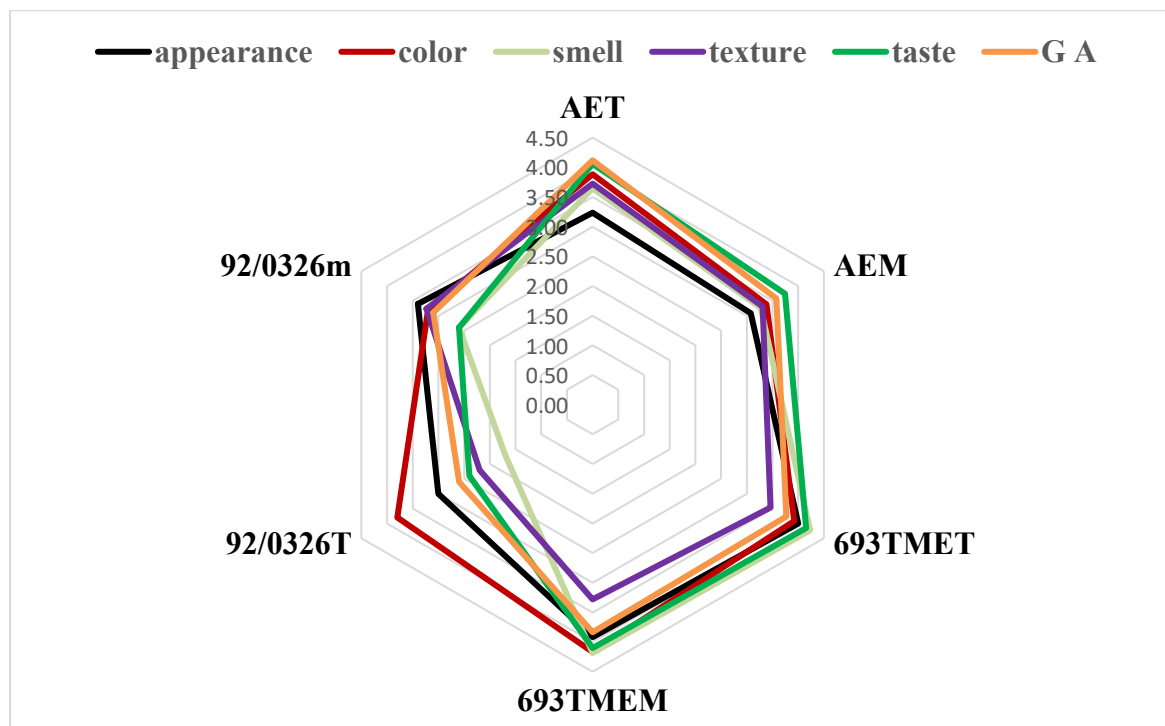


Figure 5: Impact of AM fungi inoculation and variety on hedonic properties of cassava/wheat (50/50) biscuits. AET, 92/0326T, 693TME=none mycorrhizal cassava variety; AEm, 92/0326m, 693TME= mycorrhizal cassava variety. GA=general acceptability.

IV. Discussions

Functional properties of composite flours at various substitution rates with three cassava flours from AM fungi-inoculated plants or not were analysed. The plan was to group the flours following both the cassava variety, the substitution rate, and the growing conditions. Principal component analysis of the composite flours, the cassava varieties, and the symbiotic status of the cassava plant producing the flour shows that two out of three cassava varieties show better characteristics regarding EC and SC: 693TME at all substitution rates when the flour is produced from plants inoculated with AM fungi. 92/0326 at the substitution rates (75/25 and 50/50) when the flour is produced from the plant inoculated with AM fungi (Figure 4). Food scientists use the term SC to refer to the capacity of starch granules to bind water upon heating, indicating the level of granule hydration and gelatinisation under thermal conditions (Kumar et al., 2017). In flours, the SC can be affected by factors such as variety, processing methods, particle size, substitution rate, and more (Mukhtar et al., 2025). In general, our results show a significant increase in the functional parameters studied as the substitution rate of wheat flour with cassava flour increases in both varieties, especially when the cassava flour comes from plants inoculated with AM fungi. Previous studies show that the sugar and protein content of both cassava varieties is significantly increased by inoculating the plants with AM fungi. This increase is greater in the TME 693 variety than the 92/0326 variety (Mbassi et al., 2024). This suggests that inoculation impacts plant physiology, leading to the production of these molecules. Furthermore, several other studies demonstrate the contribution of proteins and starch to the functional properties of flours (Chandra et al., 2.15; Li et al., 2023). It appears that substituting wheat flour with cassava flour improves the starch and protein content of composite flours and, consequently, parameters such as SC and EC, particularly when the involved flour comes from a cassava plant inoculated with AM fungi. The literature is consistent with the observation that SC varies with the starch content of flour (Chandra et al., 2015; Li et al., 2023).

Moreover, two out of three cassava varieties show better characteristics regarding WAC, including AET(75/25), AEM at all substitution rates; 92/0326 at the substitution rates (75/25 and 50/50) when the flour is produced from the plant, none-inoculated with AM fungi (Figure 4). This observation shows the distribution of WAC according to the variety of cassava used, the symbiotic status of the flour-producing plant and, finally, the frequency of substitution. The WAC reflects the ability of flours to bind and retain water. A significant increase ($P<0.05$) in WAC was recorded for both cassava varieties, especially from the 50/50 substitution frequency, compared to wheat flour, and between treatments for cassava flours from plants inoculated with AM fungi. Such observation could be attributed to the presence of a variable amount of starch, especially free starch in flours depending on the variety as well as the AM fungi status of the producing plant. Similar observation was also recorded while studying composite flours (Skibsted, 2015; Alviola and Monterde, 2018; Mbassi et al., 2024). The

observed variation among different flours may be attributed to differences in protein content monitored by AM fungi in plants within the growth and genotype of the cassava varieties involved, resulting to the extent of protein–water interactions, and the conformational properties of the protein molecules (Chandran et al., 2024).

The various analyses revealed that the 50/50 substitution rate encompasses a large number of functional properties. Subsequently, this substitution rate was chosen as a blended formula of wheat/cassava flours for making biscuits, and sensory analyses were conducted. Large variation depending on the cassava varieties, their AM fungi status and the sensory attributes studied was recorded (Figure 5). The AE variety shows consistency within the evaluation of sensory attributes, with a significantly high score for biscuits containing cassava flour from non-AM fungi plants. The taste attribute contributes strongly to the appreciation of biscuits of this variety, while the appearance attribute contributes less. A similar trend in sensory appreciation is observed with variety 693TME, but in this case, the texture attribute contributes the least to overall acceptability, and taste contributes the most. A researcher believes that taste attribute is a key factor which determines the acceptability of products, and highly contributes to the market success of a product (Tasnim and Suman 2015; Cardona et al., 2023). The opposite trend was observed with variety 92/0326, where biscuits containing cassava flour from plants inoculated with AM fungi were more highly rated by the panellists. Furthermore, individual sensory attributes showed very different scores for biscuits containing flour from non-AM fungi plants, and nearly homogeneous scores for flour from plants inoculated with AM fungi. These observations would provide further evidence of the genetic diversity among the different cassava varieties described by researchers in several studies. Furthermore, several studies demonstrate variation in the content of bioactive molecules between cassava varieties when inoculated with AM fungi compared to controls (Mbassi et al., 2024). The contribution of bioactive molecules to the sensory evaluation of foods, including cassava tuber-derived products, including those made with composite cassava base flour, is well established (Carocho et al., 2014; Seol et al., 2015).

V. Conclusion

This study was addressed to investigate the influence of AM fungi inoculation and the partial substitution of wheat flour with cassava flour on the functional properties of composite flours, and to do analysis of the hedonic properties of biscuits from the most promoted composite flours. The outcome of this study was that blended Formulating flour with cassava from plants inoculated with AM fungi results in an increase in the functional parameters studied compared to wheat flour. This increase is also observed between the substitute formulas with the composite flour containing flour from plants inoculated with AM fungi, compared to the control, in all varieties. Sensory analysis of biscuits made with the 50/50 substitution shows that the formula containing flour from plants inoculated with AM fungi is less appealing for varieties AE and 693TME, while the opposite is true for variety 92/0326. This study prompts a reconsideration of plant variety selection criteria, which are generally genetic, to strongly integrate nutritional parameters and especially products from processing, to better address the crucial problem of food.

Conflict of interest.

No conflict exists related to this work.

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