

**Articles for**

**ORAL PRESENTATIONS**

**SESSION 2-3**

**Post-harvest - 2**

**Effect of Sodium Bicarbonate Heated Solution on Postharvest Behavior, and Decay of Germoplasm prunes**

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**Type of presentation.** ORAL

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**Abstract.** Health and environmental concerns regarding the use of synthetic fungicides have speed-up the search for alternative approaches. The most suitable results have been achieved by combining treatments acting differently on pathogenesis. Here, we report on the behaviour of two white prune varieties ('Core' and 'Meloni') of the Sardinian germplasm as affected by an immersion treatment for 0 (control), 15, 30, 45 or 60 sec in water at 20, 50, 55 or 60 °C with or without 2% (w/v) NaHCO<sub>3</sub> (SBC). The experiment, carried out in 2008-09-10 was performed with fruit harvested at commercial maturity, treated and then stored for 1 month at 5 °C and 90% RH followed by a simulated marketing period at 20 °C and 80% RH for 6 days. Fruit weight loss, appearance, external damage, firmness and decay percentage were monitored after storage and SMP. Any of the heat treatments induced rind damage (browning or discoloration) but the overall appearance of 'Core', decreased significantly after the SMP period, especially when treated at 55 or 60 °C for 60 sec. Fruit shrivelling was the main cause of the low rating for both varieties, especially after shelf-life. SBC at 20 °C did not affect shrivelling, indicating that the heat-SBC combined treatment may be the cause of this disorder. In addition, weight loss was significantly higher in fruit treated with the salt. Immersion for 15 or 30 sec in the 2% SBC solution at 20, 50 or 55 °C was not effective in controlling decay during storage, and after shelf-life no improvement was observed compared to control. On the other hand, immersion for 45 or 60 sec in the SBC solution improved decay control at all temperatures, and best results were obtained at 50 and 55 °C. Immersions at 60 °C improved decay control, but differences were not significant compared to solutions of SBC heated at 55 °C.

KEY WORDS: GRAS compounds, *Botrytis cinerea*, crop quality, decay control.

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# Drying of Yacon Roots and Fructo-oligosaccharides Extraction After Storage Period

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**Abstract.** *Inulin is a reserve carbohydrate present in several vegetable products, formed by a chain of fructose molecules and a terminal molecule of glucose. Some vegetables has considerable inulin-type fructo-oligosaccharides amount: chicory roots (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus tuberosus* L.) and yacon (*Smallanthus sonchifolius* Poepp. & Endl.). Besides, chicory and yacon roots are highly perishable products, demanding, of the industrial point of view, a short processing period. In other words, the industry needs super-designed equipments with long inactive time during off season time. So, to allow a better design of a processing plant for obtaining fructo-oligosaccharides and for assuring a continuous supply of the product, it is necessary to study a way to make available the roots along the year. Drying is an alternative to reduce the water activity of these products and consequently to prolong their shelf life. In this project it was intended, to submit biological materials to a drying process using a vacuum oven and to evaluate the behaviour of drying. Experimental runs of yacon roots drying were accomplished intending to evaluate influence of drying operational parameters on fructo-oligosaccharides extraction. The evaluated parameters were temperature and application of vacuum pressure. Mathematical models were generated by analysis of response surface methodology. The use of higher drying temperatures resulted in decreasing of drying time. The interaction of temperature and vacuum pressure was statistically significant for fructo-oligosaccharides extraction.*

**Keywords.** hot water extraction, vacuum drying, drying rate, inulin.

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## **Introduction**

Recently it was discovered that inulin can act as substitute of sugar or of fat, with the advantage of not resulting in caloric increment (Roberfroid, Gibson & Delzenne, 1993). Besides acting as substitute of sugar or fat, inulin also presents some functional properties. It acts in organism in a similar way to dietary fibers, contributing to improve the conditions of gastrointestinal system. That substance has been used in the composition of the most several nutritious products, as breads, candies, ice creams, animal food etc.

In European countries, like Belgium, France and the Netherlands, chicory has been more used for the industrial production of inulin, mainly due to its stability in the production of long chains of glucose and fructose (GF<sub>n</sub>) and constant production, even in conditions of moderate climate. However it is possible to obtain this kind of fructo-oligosaccharide with shorter chains from yacon roots (*Smallanthus sonchifolius* Poepp. & Endl).

Yacon is an Andean plant that has tuber roots whose taste is similar to the fruits. These roots consumption has increased in Brazilian market. This root contains fructo-oligosaccharides, reason for it is known as “diet potato” in Brazil, although it is not a potato. Its moisture value reveals a perishable product and it establishes a short shelf life and short use period for the provision industry. In other words, industry needs oversized equipments with long inactive time during off-season time. In this way, drying appears as an alternative to enlarge period of storage of this product and to assure a continuous supply of the product along the year.

In this research work, yacon roots were submitted to vacuum drying process in order to evaluate the effects of operational parameters on behavior of the drying process. In addition, the dried roots were submitted to a hot water process. Then, the influence of drying operational parameters was evaluated on the amount of extracted fructo-oligosaccharides.

### ***Vacuum Drying***

In vacuum drying, the product is heated up by steam, for conduction or by radiation, while it stays under conditions of low pressure. That drying process offers a series of advantages as, for instance, a low drying temperature and low oxidation.

Drying of biological thermo-sensitive products is a complex process that can affect their biochemical (viability, atrophy of the cells), enzymatic (stability, catalytic ability), chemical (nutritional value decrease, degradation of vitamins), physical (solubility, rehydration, shrinking, aroma loss) and mechanical properties (elasticity, hardness, friableness, fragility) (PAN et al., 1999). For thermo-sensitive materials, the vacuum dryers should be used in the reduction of drying temperature.

Since vacuum drying is usually executed at low pressures, water is evaporated in low temperatures; consequently, drying can be accomplished in low temperature. Several researchers combined the advantages of drying by infrared with vacuum drying in order to dry several food products (Swasdisevi et al., 2007). Besides, vacuum drying offers a fast potential drying with low consumption of energy, compared with other methods.

### ***Hot Water Extraction***

Diffusion in hot water is a well-known process to extract substances from solid products, and later the substance can be separated from water in different manners, as spray drying.

## Material and Methods

Yacon roots were purchased from same supplier in city of Campinas, Brazil. Until the moment of using them, yacon roots were stored in cold room at approximately 17°C. The roots were cut in cube format of about 1 cm with appropriate manual device, before drying process.

The drying process was accomplished in vacuum oven (model Q-819V2, Quimis). The temperature range of vacuum oven chamber varies from 25°C to 200°C, while the operational range of vacuum pump is between 0.01MPa and 0.098MPa.

Drying data were obtained by consecutive weighting of samples. After finishing drying process, a sample of dried root was used for determining dry mass according to methodology proposed by AOAC (1998). The drying operational parameters followed a central composite experimental design (Table 1). Temperature and vacuum pressure were the independent variables.

Table 1. Experimental design and responses for drying of yacon roots and extraction of inulin.

Run	Coded variables		Actual variables		Drying rate	Effective diffusivity	Extracted soluble solids
	X <sub>T</sub>	X <sub>P</sub>	T	P	dX/dt	D <sub>eff</sub>	SS
1	-1	-1	50.0	0.060	0.0146	1.00 x 10 <sup>-9</sup>	1.035
2	-1	1	50.0	0.090	0.0228	7.83 x 10 <sup>-10</sup>	0.456
3	1	-1	90.0	0.060	0.0619	2.43 x 10 <sup>-9</sup>	0.591
4	1	1	90.0	0.090	0.0807	4.19 x 10 <sup>-9</sup>	0.660
5	-1.41	0	41.7	0.075	0.0057	7.14 x 10 <sup>-10</sup>	0.645
6	1.41	0	98.3	0.075	0.0433	3.38 x 10 <sup>-9</sup>	0.729
7	0	-1.41	70.0	0.054	0.0250	1.09 x 10 <sup>-9</sup>	0.748
8	0	1.41	70.0	0.096	0.0310	1.02 x 10 <sup>-9</sup>	0.719
9	0	0	70.0	0.075	0.0275	7.96 x 10 <sup>-10</sup>	0.690
10	0	0	70.0	0.075	0.0256	8.09 x 10 <sup>-10</sup>	0.541
11	0	0	70.0	0.075	0.0329	7.10 x 10 <sup>-10</sup>	0.742

In case of drying process, mean drying rates and effective diffusivities were the evaluated responses. These values indicate the velocity of decrease of material moisture content. Mean drying rates were determined by relation between decrease of moisture content and increment in time. The effective diffusivities were obtained by fitting dimensionless moisture content curve based on Second Fick's Law by STATISTICA software (Statsoft Inc., 2008). The Response Surface Methodology – RSM was also evaluated by STATISTICA software by Analysis of Variance (ANOVA) with a confidence level of 95%.

Both material obtained from drying and extraction were stored in cold chamber with temperature less than 5°C for approximately 6 months.

Samples originated from drying experimental runs were the same samples which were used for extraction of inulin. So, the independent variables were the same of drying process. This procedure was performed in order to evaluate the effects of drying on the extraction of inulin. The amount of extracted soluble solids which was taken as indirect measurement to the concentration of inulin. For each test, the samples were weighted according to their water content and relation water mass:root dry mass (12.0 g<sub>H2O</sub>/g<sub>DM</sub>). Subsequently, a defined mass of dried roots was immersed in distilled water in a beaker (250 ml) and placed in a circulated heated bath with fixed temperature. Parameter values for water:root mass ratio, time and temperature of the bath were defined previously (Oliveira, 2005).

## Results and Discussion

Drying process can be evaluated during the periods of constant and falling rate. Constant rate period of drying is hardly observed in biological material, and the presence of this period shows that the material has high water content. Yacon is one of biological products that permit to observe the constant rate period. The mean drying rates and effective diffusivity values related to constant and falling rate period, respectively, and extraction results are shown on Table 1.

### Constant rate period analysis

The constant rate period does not occur so frequently for biological materials. However, in cases with high moisture content, this type of behavior can be observed at the beginning of drying process. Oliveira (2009) found this period, studying vacuum/infrared radiation drying. Yacon has high moisture content, which is essential for observation of constant rate period of drying. Statistical analysis provides an equation that determines drying rate as function of temperature, which was the only parameter statistically significant (Equation 1 and Figure 1):

$$\frac{dX}{dt} \left[ \frac{g_{H_2O}}{g_{DM} \cdot \text{min}} \right] = 0.033724 + 0.039581 X_T \quad (1)$$

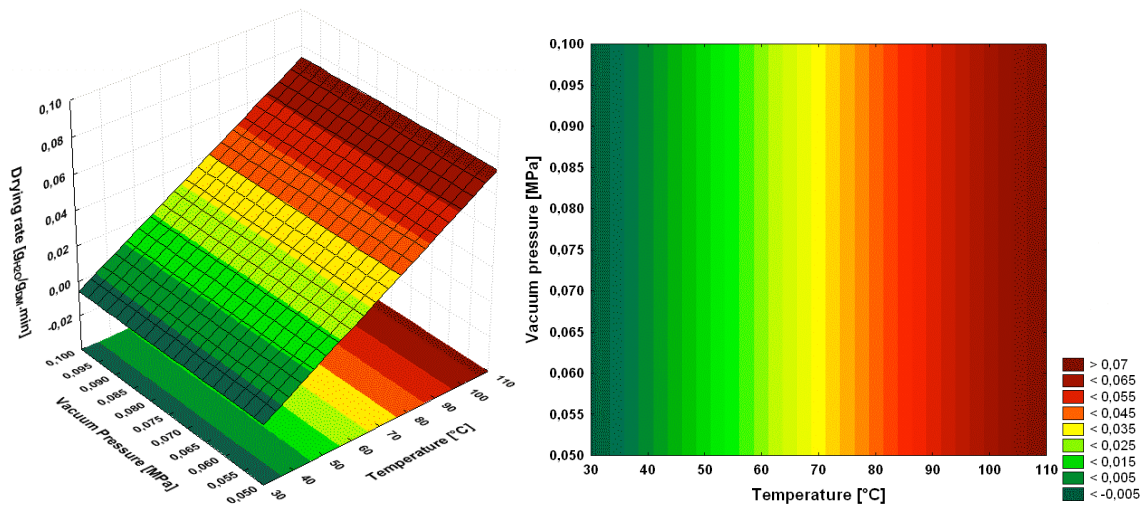


Figure 1. Response surface and contour plot for drying rate of yacon roots drying.

### Falling rate period analysis

The values of effective diffusivity were determined by the Fick's second law, and the mathematical models from Fick's law describe an approximation for the drying kinetic behavior related to falling drying rate period. The mathematical model which describes effective diffusivity values in function of operational parameters are shown in Equation 2 and Figure 2.

$$D_{eff} \left[ \frac{m^2}{s} \right] = 7.72 \times 10^{-10} + 1.08 \times 10^{-9} X_T + 7.75 \times 10^{-10} X_p + 1.80 \times 10^{-10} X_T^2 + 2.79 \times 10^{-10} X_p^2 + 4.94 \times 10^{-10} X_T X_p \quad (2)$$

Swasdisevi et al. (2007) studied the drying of slices of bananas in dryer to combined vacuum with heating for distant infrared radiation. Those authors showed that the drying rates increased with the decrease of the absolute pressure of drying chamber. The effect of the vacuum level in the behavior of drying of banana slices was more pronounced to lower temperatures.

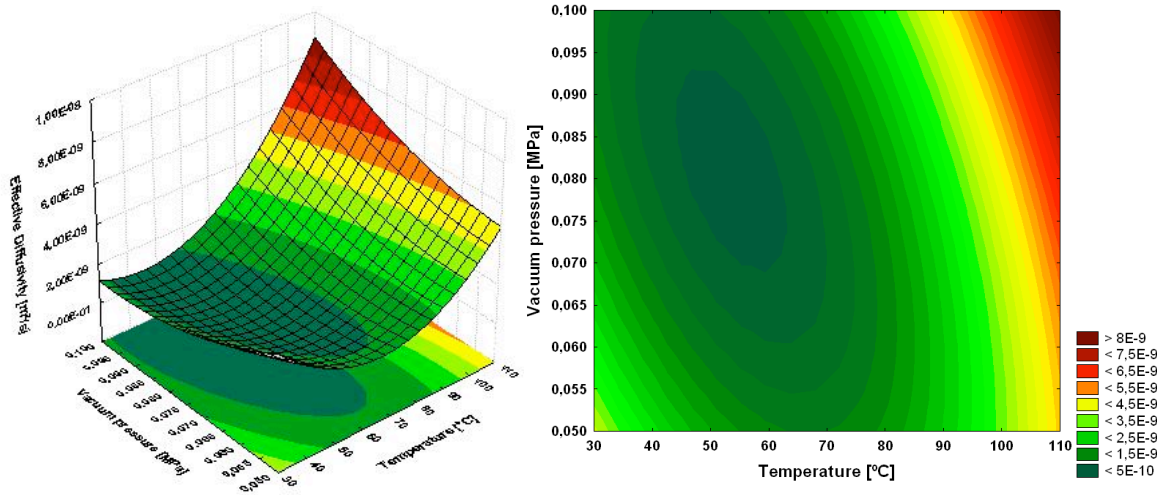


Figure 2. Response surface and contour plot for effective diffusivity of yacon roots drying.

### Extraction

The influence of the operational drying conditions on the amount of extracted soluble solids was evaluated by building a response surface of this parameter as function of temperature and negative pressure on drying chamber. The model that best represented extracted soluble solids amount is given by Equation 3, shown in Figure 3.

$$SS \left[ \frac{g_{SS}}{g_{DM}} \right] = 0.7960 + 0.0208 X_T X_P \quad (3)$$

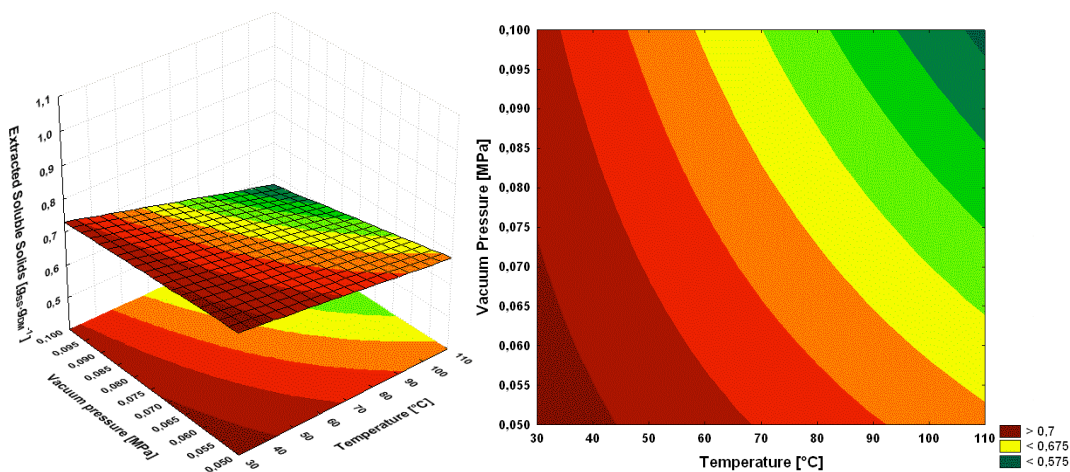


Figure 3. Response surface and contour plot for extracted soluble solids of dried yacon roots.

It is noticed that only interaction between independent variables was considered statistically significant. This effect has negative influence on the amount of extracted soluble solids, i.e., the increase of these variables results in a decrease of extracted soluble solids. While the increase of the variables increases drying rate and effective diffusivity in drying process, the same increase of variables results in a decreased of extracted soluble solid. Possibly, it is necessary to admit a slower drying in order to avoid loss of soluble solids.

## Conclusions

The present work determined the values of effective diffusivity and mean drying rate on the drying process of yacon roots. Checking literature, drying rates and effective diffusivities have to change with different temperatures and negative pressures, as observed in this research work. Increasing the values of temperature and vacuum pressure causes the consequent increase of effective diffusivity and drying rate. Extraction process was accomplished intending to evaluate influence of drying operational parameters on fructo-oligosaccharides extraction. Interaction of temperature and application of vacuum pressure was significant.

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## Nomenclature

$D_{\text{eff}}$	effective diffusivity	[m <sup>2</sup> /s]
$dX/dt$	drying rate	[g <sub>H<sub>2</sub>O</sub> /g <sub>DM</sub> .min]
P	vacuum pressure	[MPa]
SS	extracted soluble solids	[g <sub>SS</sub> /g <sub>DM</sub> ]
T	temperature	[°C]
$X_P; X_T$	coded variables for vacuum pressure and temperature	[dimensionless]

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# Development of a rice cleaner - grader

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**Abstract.** *Locally processed rice in Nigeria has been known to be highly nutritious, however the physical quality of the milled rice is usually impaired with broken grains and impurities.*

*A three sieve cleaning and grading machine was constructed and tested at a fan speed of 240 r.p.m.. Aerodynamics and physical characteristics of three varieties of rice was determined and were used for design considerations for the construction of the equipment- An air blast of 4.8ms<sup>-1</sup> was utilized to separate light materials while apertures diameter of 3, 5.5 and 7mm were selected for bottom, intermediate and top sieves to effect grading of whole kernels. Milled rice fed into the machine was tested at tilt angles of 4° for the intermediate sieve and at blower inclinations of 0° and 5°.*

*The spherical mean ranged from 2.85 to 3.45mm and sphericity existed within 0.41mm to 0.50. and terminal velocity was from 4.81 to 6.36ms<sup>-1</sup>. The cleaning efficiency of the machine ranged from 63.97 – 85.382% with the highest obtained in FARO 44. Efficiency of grain grading for parboiled milled rice was from 71.78 to 94.29% and the highest was obtained in ITA 150 at a blower inclination of 5°. Machine capacity ranges from 1000 – 1,200kg/hr The incorporation of the grading machine after milling has upgraded locally processed rice quality from grade III to grade II. Better machine performance was obtained at blower inclination of 5° except for FARO 52*

**Key words.** Rice, tilt angle, sieves and grading machine

## Introduction

Rice is a structural component of Nigeria diet and is a widely grown crop because of its economic importance. Rice consumption has increased steadily over the last decade as a result of population growth and rapid urbanization. Locally produced rice have been known to be highly nutritious, however, the quality of its milled rice are usually poor. They are characterized with high percentage of impurities like husk and stones as well as under size / broken grains which reduces its economic value and makes it difficult to compete favorably with imported ones. The low level technology input in rice production has contributed to high grain losses and poor quality of Nigerian rice, thereby limiting sustainable food chain. According to Ogunlowo and Adesuyi, 1999: the demand for consumption of locally produced rice is low. This has been attributed to poor harvesting and post harvest handling method which encouraged the presence of contaminants such as stones, stick, chaff and leave stalk

Milled rice obtained from the processors are usually winnowed by hand, separating grains from contaminants – materials other than grains (MOG) This procedure is tedious and time-consuming and a lot of dockages, small stones and broken remains mixed with the rice in the end. The rice has not been graded and they are not well cleaned. This explains why local rice available in the market is characterized with high percentage of breakages and impurities which attracts low price. A cleaner / grader need to be incorporated for further cleaning after milling operations.

Henderson and Perry (1976) stated that mechanical methods of separating rice from foreign materials depend on the difference in such characteristics as size, shape and specific weight. Harrison and Blecha (1983) suggested that orientation of particles as well as combined effect of frequency and amplitude of oscillation, the screen slope and hanger angle and the friction between particles and screen should be considered for screen sizing. Grain size determination is important in the design of screen opening. Moshenin (1986) suggested that grains can be best assumed spherical and their spherical mean can be obtained by taking geometric mean of their mutually perpendicular axes and sphericity is the percentage of ratio of the spherical mean and the major diameter. When the value of sphericity is greater than 85% circular shaped sieves is sufficient for separation while those that within 32 to 50% require an oblong shaped sieve for final separation. Ogunlowo and Adesuyi (1999) accomplished separation of stones and other contaminants with a combination of oscillating sieves variable directional air stream at 160 r.p.m. Tabatabaefar et al (2004) designed and developed a second sieving and grading machine for chick pea which attained an overall cleaning efficiency of 84% with a use of four sieves vibrated at 200 r.p.m. Also Okunola and Igbeka (2009) improved on an appropriate technology cereal cleaner Igbeka (1984) and obtained a higher cleaning efficiency of 71% for paddy than 61% when vibration occurred at 240 r.p.m. instead of 250 r.p.m.

## Materials and Method

This design adopted some concept as suggested by Kewal (1976) that separation and cleaning should use a continuous force field rather than a gravitational force and must be accompanied within a minimum space. Also a positive means of supplying kinetic energy to the crop material is required to loosen the bulk kernels which are firmly lodged inside the straw mass.

30 paddy rice samples were randomly selected from the various rice varieties and the axial dimension of three axes (major, intermediate and minor axes) were measured using a digital vernier caliper. The data was used to calculate the estimated spherical mean as suggested by Moshenin (1986). The average dimension of the whole grains of paddy randomly picked for major diameter ( $L$ ), intermediate diameter ( $W$ ) and minor diameter ( $T$ ) were used for the following:

$$\text{The Geometric mean } D; D = (L \times W \times T)^{1/3} \quad (1)$$

$$\text{The sphericity of paddy } \theta (\%); \theta = \frac{(L \times W \times T)^{1/3}}{L} \times 100 \quad (2)$$

Geometric characteristics of milled grains and the various contaminants have been determined with a view to effect separation. Light weight materials shall be blown by air blast, while heavier straws and stalk slide over the top and intermediate screens as over tailings. Whole grains shall be separated from stones and broken grains by bottom screen inclination and vibrations.

The terminal velocity of the three varieties of rice was determined with a wind column apparatus.

### ***Design for cleaner / grader***

The cleaner/grader was designed to be a portable electric motor driven to grade milled rice into whole grain and broken grains which shall be collected in different receptacles and will also blow away light impurities with the aid of air blast from the blower. The machine had been designed using relevant engineering principles and theories. Fig. 1 shows the schematic diagram of the cleaner – grader.

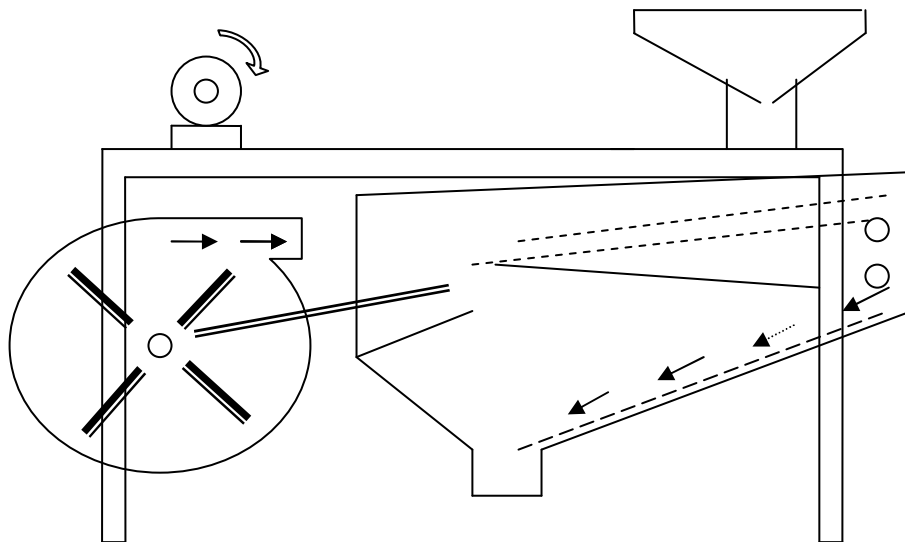


Fig. 1 Schematic diagram of a rice cleaner / grader

The theoretical power requirement for oscillation of the sieve assembly can be approximated by summation of power required for the movement in the vertical and

horizontal direction and was determined to be 1.52Hp using the approach of Okunola and Igbeka (2009)

### **Screen mechanism**

The basic requirement for grain separation is that there must be grain movement on the sieve surface towards the lower end of the sieve in a vibrational mode. The sieve to and fro motion is effected by an eccentric connection to power source. The sieves as a result of eccentric drive connection to the casing experiences horizontal oscillation and small vertical motion. The combined motion ensures that the seed move or slides down from the screen and at the same time hops up to give the whole bulk a stir. Screen and hanger angle are provided to ensure efficient separation.

### **Cleaning and grading**

The product from the milling was fed into the cleaner / grader for further separation this machine vibrates it's sieve assembly which contains three sieves of aperture sizes diameters of 3, 5.5 and 7mm at 240 r.p.m. The bottom, intermediate and top sieve tilt angles were maintained at 15,4 and 2° respectively to determine extent of cleaning during the experiment, however the blower inclination was varied from 0 to 5° to the horizontal. All the sieves were fixed inside the sieve casing suspended by four hangers and receive oscillating motion by eccentric drive obtained from the fan shaft. Milled rice were fed into the hopper with a gate opening set at 6mm to allow for effective distribution and sufficient resident time over the sieves for through separation and grading. The milled rice was sorted into four categories, namely; light materials away from the top sieve, over tailings sliding over the lower ends of the two upper sieves, brokens / undersized / small stones and whole kernels. Final separation of the various rejects and product obtained from the receptacles into various quality grades were done with a manually operated 2mm square sieve mesh. Grains with 3/4 or more length were regarded as whole kernel while those shorter were taken as brokens Sampang (2005). For purposes of evaluation, the following terms and nomenclature were adopted for the separation efficiency as used by Igbeka (1984). Method for separation processes is shown in equations 3, 4, 5 and 6.

1. Efficiency of separating whole grain -  $E_{GR}$

$$E_{GR} = \left( \frac{GP}{GP + GR} \right) \times 100 \quad 3$$

2. Efficiency of separating materials other than grain (MOG) -  $E_{BC}$

$$E_{BC} = \left( \frac{BR}{BR + BP} \right) \times 100 \quad 4$$

3. Cleaning Efficiency

$$E_T = \frac{E_{GR} \times E_{BC}}{100} \quad 5$$

#### 4. Efficiency of grading of whole grain in products

$$E_G = \left( \frac{GP}{GP + BP} \right) \times 100$$

6

### Results and discussion.

Grain classification technique as used by Tabataeefar et al (2004) was adopted to group all the spherical means of the parboiled milled rice. This is shown in Table 2 and Microsoft excel was used to determine the overall spherical mean to be 3.05mm. and a 3.00mm diameter aperture with 85% coefficient of opening was selected for the bottom sieve of the grader to separate whole kernels from broken / undersized ones.

Table 1 Classification of all varieties of parboiled milled rice

Spherical Mean (mm)	Frequency	Cumulative (%)
2.60	2	1.11
2.80	24	14.44
3.00	54	44.44
3.20	51	72.78
3.40	37	93.33
3.60	11	99.44
3.80	1	100.00

### Machine efficiency

Cleaned milled rice and materials other than whole grains were collected at various receptacles, weighed and recorded and average of three replicates obtained. Using equations 4, 5 and 6 cleaning and grading efficiencies were shown in Table 2

Table 2 Summary of weights collected during cleaning and grading with machine efficiencies

Variety	FEED (g)	WG (g)	BG (g)	GP (g)	BP (g)	GR (g)	BR (g)	E <sub>GR</sub> (%)	E <sub>BC</sub> (%)	E (%)	E <sub>G</sub> (%)
<b>Blower inclination at 0°</b>											
ITA 150	1600	776.4	362	731.1	121	63.2	684.7	92.04	84.98	78.22	85.80
FARO 44	1600	897.5	232	865.4	93.2	32.5	599.9	96.38	86.55	83.42	90.28
FARO 52	1600	704.9	389.6	681.4	268.6	23.5	626.8	96.67	70.00	67.67	71.73
<b>Blower inclination at 5°</b>											
ITA 150	1600	798	358.6	752.4	101.5	45.6	700.5	94.29	87.34	82.35	94.29
FARO 44	1600	895.6	168.2	861.8	79.4	33.8	625	96.23	88.73	85.38	91.56
FARO 52	1600	753.6	287.5	730	260.5	23.6	585.9	92.41	69.22	63.97	73.70

WG Whole grain; BG Broken grain; GP Good product; BP Bad product; GR Good reject; BR Bad reject; E<sub>GR</sub> Efficiency of grain separation; E<sub>BC</sub> Efficiency of brokens -chaff separation; E Cleaning efficiency; E<sub>G</sub> Efficiency of grain grading

### Conclusion

Variety has significant effect on size characteristics of milled rice. The cleaning / grading equipment has been designed to improved physical quality of Nigerian milled rice and should be incorporated in rice processing at cottage industry. The cost of production of the

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prototype was 850USD. Commercial production of this cereal cleaner will be viable as they shall be utilized by the small scale rice processor in the country.

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ABSTRACT N°442 - CIGR Section 6 – NANTES – APRIL 2011

## Weather Effects on Quality and Safety of Ready-To-Eat Leafy Greens in the Desert Southwest, USA

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**Abstract.** *For several years we have investigated effects of weather, and subsequent alteration in plant physiology, on quality and safety of leafy greens during a November-April season in the Sonora desert, North America. A three-year water survey in the Yuma Valley, Arizona, where great part of the US winter leafy greens are produced, has shown that quality of irrigation water varies significantly throughout the year. In general, presence of bacteria indicators in the water declines dramatically during the winter coinciding with lower temperatures. Other environmental factors did not correlate with common indicators. Irrigation water (quality and application fashion) was shown to be a critical variable on the microbial population of leafy greens, which may affect both pathogen and competitive bacteria survival. While winter months showed lower levels of fecal coliforms overall, the potential for extended survival of a pathogen in the field during these months was greater, declining during Spring and Summer months. Higher microbial population was observed after a rain event. The survival of common pathogens at different “water qualities” also showed differences, with higher salinity in the water resulting in more rapid decline of pathogens, even within salinity levels tolerated by leafy greens. Aiming to estimate shelf life of selected ready-to-eat leafy greens we analyzed different variables that could be used as predictors such as fresh weight, water potential, moisture content, degree days, light intensity. A discussion of the results is provided, spinning around the major fact that excess moisture at harvest (and/or excessive growth rate) negatively affects quality during postharvest storage.*

**Keywords.** Postharvest, water potential, water activity,

# Studies on Accumulation Mechanism of $\gamma$ -Aminobutylic Acid in Tomato Fruits under Modified Atmospheres

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**Abstract.** *Modified atmospheres have been found to be effective for accumulating  $\gamma$ -aminobutylic acid (GABA) in tomato fruits. Therefore, the accumulation mechanism of GABA under modified atmosphere was attempted to be disclosed in the current study. Glutamate decarboxylase (GAD) and GABA transaminase (GABA-TK) activities were slightly promoted and considerably depressed under modified atmosphere, respectively. GAD and GABA-TK work for production and degradation of GABA, respectively. Therefore, we concluded that accumulation of GABA in tomato fruits is caused by the changes in activities of enzymes associated with GABA shunt under modified atmospheres.*

**Keywords.**  $\gamma$ -aminobutylic acid, tomato fruits, *Solanum lycopersicum* L., modified atmosphere

## Introduction

$\gamma$ -aminobutylic acid (GABA) is an inhibitory amino acid that is widely distributed throughout the biological world (Kinnersley & Turano, 2000). It has been reported to have relaxation and immunity enhancement effects (Abdou et al., 2006), as well as antihypertensive and natriuretic effects (Yamakoshi et al., 2007).

GABA is mainly produced by glutamate decarboxylase (GAD) (EC 4.1.1.15) reaction catalyzing decarboxylation from glutamate (Bouché & Fromm, 2004). This suggests that production of GABA is promoted in organisms with a high concentration of glutamate as a main substrate of the enzymatic reaction. In the current study, tomato (*Solanum lycopersicum* L.) fruits were selected for stimulating GABA production because it contains high glutamate levels (Liu and Luh, 1979).

Previous reports have shown that high levels of GABA accumulate rapidly in plant tissues exposed to a variety of different stresses (Kinnersley et al., 2000). Stress from anoxia is reported to be effective for stimulating the production of GABA (Kinnersley et al., 2000); however, horticultural products stored under anoxia / hypoxia conditions generate off-odors during ethanol fermentation and lose their commercial value (Kader, 1986). Makino et al. (2008) reported that GABA production in tomato fruits was stimulated by storing 6 d at 30°C under an aerobic condition including 11% O<sub>2</sub> and 9% CO<sub>2</sub>. Mae et al. (2010) reported that the production was stimulated by storing 7 d at 25°C under an aerobic condition including 10% O<sub>2</sub> and 12% CO<sub>2</sub> as well. However, the mechanism stimulating GABA production under the modified atmosphere has not been researched.

The objective of this study was to explain the relationship between atmosphere around tomato fruits and dynamic changes in compounds associated with GABA shunt on the basis of concentrations of the compounds and enzymatic activities.

## Materials and methods

Vine-ripened tomato fruits (Momotaro cultivar) were harvested on June 30, 2010, at a farm in Wakayama Prefecture, Japan. After harvesting, the samples were maintained at 10–16°C, transported to the laboratory within 1 d, and stored in a constant temperature unit (15°C) for another 1 d before use. The average mass of the 96 pieces of fruit was 0.193 kg (range 0.166–0.231 kg). Intact fruits without any pretreatments were used for the following experiments.

Three-layer laminated (polyethylene terephthalate (PET)/aluminum/polyethylene) high barrier pouches Lamizip<sup>®</sup> (AS ONE Corporation, Tokyo) (O<sub>2</sub> permeance: 1.0 mL·m<sup>-2</sup>·d<sup>-1</sup>·atm<sup>-1</sup>, film thickness: 110 μm; surface area: 0.094 m<sup>2</sup>) were used to create hypoxic conditions with/without high CO<sub>2</sub> levels around the tomato fruits. Micro-perforated PET/low-density polyethylene (LDPE) film pouches (O<sub>2</sub> permeance: 8,000 and 13,000 mL·m<sup>-2</sup>·d<sup>-1</sup>·atm<sup>-1</sup>, film thickness: 47 μm; surface area: 0.085 m<sup>2</sup>) were used to contain a modified aerobic atmosphere with/without high CO<sub>2</sub> at 25°C. Macro-perforated pouches (the same PET/LDPE film as the micro-perforated pouch with 5 mm $\varnothing$  × 8 holes) were used as a control in which the in-package atmosphere was expected to be the same as that of ambient air.

Two tomato fruits were sealed in a pouch containing 10 g of C<sub>2</sub>H<sub>4</sub> absorbent (Picón et al., 1993) using an impulse sealer. Ten grams of the CO<sub>2</sub> absorbent Ageless C<sup>™</sup> (Mitsubishi Gas Chemical Company, Inc., Tokyo) were also sealed in the Lamizip<sup>®</sup> and 13,000 mL·m<sup>-2</sup>·d<sup>-1</sup>·atm<sup>-1</sup> pouches to control CO<sub>2</sub> at 0%. The prepared pouches were stored at 25°C for 7 d in the dark at a constant temperature. Aliquots (300 μL) of in-package gas were sampled over time using a

gastight syringe inserted through a silicone rubber septum on the pouch. Oxygen and CO<sub>2</sub> concentrations in the sampled gas were measured by gas chromatography according to the procedure reported by Makino et al. (2008).

Tomato fruits were sampled over time for chemical analysis. Changes in concentrations of compounds and in enzymatic activities in all tomato samples were measured according to the method of Akihiro et al. (2008).

JMP® 8.0.2 software (SAS Institute Inc., Cary, USA) was used for statistical analysis. When between-class variation was significant at  $P < 0.05$  using one-way analysis of variance (ANOVA), mean values were compared by the least significant difference (LSD) test ( $P < 0.05$ ).

## Results and discussion

The in-package atmosphere surrounding the tomato fruits at steady state was presented in Table 1.

Table 1: In-package atmosphere surrounding tomato fruits at steady state (25°C).

Pouch	C <sub>2</sub> H <sub>4</sub> absorbent	CO <sub>2</sub> absorbent	O <sub>2</sub> , %	CO <sub>2</sub> , %
Three-layer laminated	+	–	2	40
Three-layer laminated	+	+	2	0
Micro-perforated	+	–	10	12
Micro-perforated	+	+	10	0
Macro-perforated	+	–	21	0

Atmosphere effective for stimulating GABA production at 25°C (Mae et al., 2010) was created using micro-perforated pouches. Therefore, the atmospheres presented in Table 1 may be valid for investigating the relationship between atmosphere around tomato fruits and dynamic changes in compounds associated with GABA shunt (Figure 1).

GABA concentrations in tomato fruits in three-layer laminated and micro-perforated pouches after 7-d storage were kept at same levels as those on 0 d and significantly higher than those in the air (macro-perforated pouches). However, GABA concentration was not dependent on the existence of CO<sub>2</sub>.

Activities of GAD and GABA-transaminase (GABA-TK, EC 2.6.1.19) in preparations from tomato fruits sealed in three-layer laminated and micro-perforated pouches were higher and lower than those in the air, respectively. The amounts of mRNA for GAD in tomato fruits sealed by packaging materials were also higher than those in the air. GAD catalyzes the production of GABA from glutamate (Bouché & Fromm, 2004). Therefore, stimulation of GABA production was caused by the increase in induction of GAD. GABA-TK catalyzes the decomposition of GABA to glutamate and  $\alpha$ -ketoglutarate (Bouché & Fromm, 2004). Therefore, reduction of GABA-TK activity will be associated with retention of GABA concentration. On the other hand, there was a positive correlation between GABA and succinic acid concentrations. Accumulation of succinic acid may contribute to the feedback inhibition against GABA-TK. When succinic acid is oxidized to fumaric acid in the tricarboxylic acid cycle, flavin adenine dinucleotide (FAD) is reduced to FADH<sub>2</sub> that finally react with O<sub>2</sub> at the terminal of respiratory chain (Makino et al., 1996). The reduction of O<sub>2</sub> around tomato fruits may cause the accumulation of FADH<sub>2</sub> and the depression of the oxidation of succinic acid.

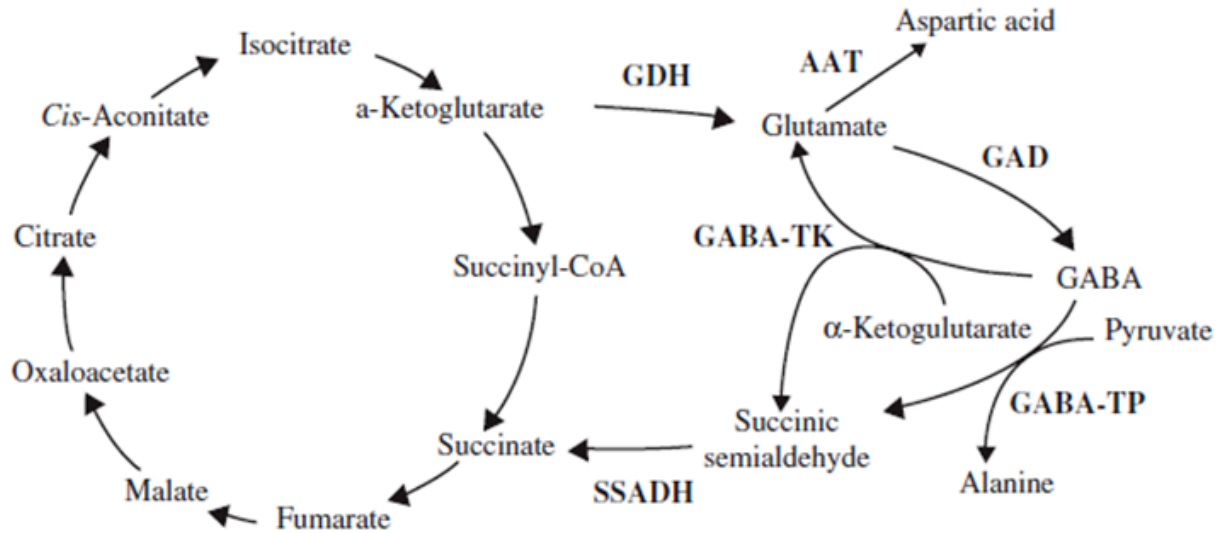


Figure 1.  $\gamma$ -Aminobutylic acid shunt

## Conclusions

Accumulation of GABA in tomato fruits under modified atmosphere was caused by the increase in GAD derivation and reduction of GABA-TK activity.

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