Flor Yeast Proteomic Response to the Lack of Fermentable Carbon Source

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ABSTRACT

Flor yeasts metabolism highly configures organoleptic properties in Sherry type wines during biological aging (Peinado et al., 2009). Along this process, the mentioned yeasts prevail by forming a biofilm in the wine surface and the presence of certain proteins involved in the response to stresses becomes essential for survival, among the most important ones: lack of fermentable carbon sources. The main aim of this study is to compare proteome data from the flor yeast *Saccharomyces cerevisiae* G1 in a typical biological aging condition (without fermentable carbon source) and in a fermentative condition (with fermentable carbon source). In this work, OFFGEL fractionator coupled to LTQ Orbitrap XL MS equipment were used trying to identify the maximum possible number of proteins related to the lack of fermentable carbon source stress in a synthetic biological aging and under a reference fermentative condition. As a result, 18 stress proteins responding to the lack of fermentable carbon source have been detected only in the biological aging condition or higher than in the reference condition which are involved in biofilm formation, perception of stimuli among other biological processes. This study together with other experiments that definitively confirm the necessity of these proteins synthesis, may lead to the genetic improvement of flor yeast strains aimed to enhance their survival during the biological aging process and hence the whole wine elaboration process.

Keywords: *Flor yeast, lack of fermentable carbon source, proteome.*

INTRODUCTION

Sherry is a fortified wine made from white grapes that are grown near the town of Jerez de la Frontera in Andalucía, Spain; but also produced in other areas worldwide. It is characterized by a specific color, texture, flavour and taste; consequence of a process known as “Biological Aging” which is carried out during the wine elaboration procedure after the must fermentation. Along this and because of the metabolism performed by a special type of biofilm-forming yeast strain, so-called flor yeast, ethanol and glycerol are consumed while other chemicals,

like acetaldehyde or acetic acid, are produced (Peinado and Mauricio, 2009).

Flor yeasts have to face several harsh conditions for survival (Alexandre, 2013). One of the principal is the lack of a fermentable carbon sources like glucose or fructose already consumed during the previous fermentation process. Zara et al., (2010) considered the formation of the biofilm (also called “flor” or “velum”) an adaptation system of the flor yeast to a medium which lacks a fermentable carbon source. Biofilm formation in the wine-air area allows the yeast to reach an

oxygen-rich zone where it is possible to catalyze ethanol or glycerol, both produced in fermentation.

Until now, genes that codify stress response proteins have been used as targets for the genetic improvement of wine yeast strains to enhance the fermentation performance (Pretorius et al., 2002) although their use in the wine production is forbidden almost in totality. Also, in the biological aging process, a higher quality in velum and a higher cell viability have been attained by the overexpression of response to stress genes (Fidalgo et al. 2006; Zara et al. 2009; Fierro-Risco et al., 2013).

In order to detect proteins related with the flor yeast response to a medium without a fermentable carbon source, a proteome analysis have been carried out from a flor yeast strain grown in a biological aging condition (BAC) without fermentable carbon source, and in a reference fermentative condition (RFC) (with fermentable carbon source) for comparison and a database search has been performed in order to detect proteins involved in the response to lack of fermentable carbon source.

MATERIALS AND METHODS

Flor yeast *Saccharomyces cerevisiae* G1 strain (ATCC: MYA-2451), was used in this work. A population of 1×10^6 cells/mL was inoculated in a biofilm formation medium or BAC (0,67% (w/v) YNB without amino acids (Difco), 1% w/v glycerol, 10 mM of glutamic acid and 10% (v/v) ethanol added after sterilization, without shaking during 29 days) and in the non-biofilm formation medium or RFC (0,67% (w/v) YNB without amino acids (Difco), 17% glucose and 10 mM of glutamic acid shaking during 12 hours). The process was carried out at 21 °C. All media were autoclaved at 120 °C for 20 minutes. Cells from the biofilm were harvested by suction and from the non-biofilm culture by centrifugation both in an initial phase when the cell viability was higher than 90%. The resulting cellular pellet from each condition was resuspended in 1 mL extraction buffer supplemented with Protease Inhibitor Cocktail tablets, and cell wall was broken by vortexing in a Vibrogen Cell Mill. Glass beads as well as cell debris were discarded by centrifugation. Protein precipitation was carried out by overnight incubation at -20 °C after addition of 10% w/v of trichloroacetic acid (TCA) and 4 volumes of ice-

cold acetone to the supernatant. After incubation, samples were centrifuged and the protein pellet was vacuum dried and then resuspended in solubilization buffer (8 M urea, 2 M thiourea, 4% CHAPS and 1% DTT). Protein concentration was estimated by Bradford assay (1976) and samples stored at -80 °C until proteins analysis.

The OFFGEL High Resolution kit pH 3–10 was used for protein preparative isoelectric focusing (IEF) in solution. Protein samples ($450 \pm 50 \mu\text{g}$) were solubilized in Protein OFFGEL fractionation buffer, glycerol, and buffer with ampholytes and aliquots evenly distributed in a 12-well 3100 OFFGEL Fractionator tray. Preset program OG12PR00 separation limits were used following recommendations of the manufacturer: 4500 V, 200 mW, and 50 μA ; starting voltage, 200–1500 V; ending voltage, 5000–8000 V; after the application of 20 kVh, the protein separation zones were maintained at constant voltage. Peptides from each well were scanned and fragmented with the LTQ Orbitrap XL mass spectrometer equipped with a nano LC Ultimate 3000 system. The electrospray voltage was set to 1300 V and the capillary voltage to 50 V at 190 °C. The LTQ Orbitrap was operated in the parallel mode, allowing for the accurate measurement of the precursor survey scan (400–1500 m/z) in the Orbitrap selection, a 60000 full-width at half-maximum (FWHM) resolution at m/z 400 concurrent with the acquisition of three CID Data-Dependent MS/MS scans in the LIT for peptide sequence, followed by three Data-Dependent HCD MS/MS scans (100–2000 m/z) with 7500 FWHM resolution at m/z 400 for peptide sequence and quantification. The normalized collision energies used were 40% for HCD and 35% for CID. The maximum injection times for MS and MS/MS were set to 50 ms and 500 ms, respectively. The precursor isolation width was 3 Da and the exclusion mass width was set to 5 ppm. Monoisotopic precursor selection was allowed and singly charged species were excluded. The minimum intensity threshold for MS/MS was 500 counts for the linear ion trap and 8000 counts for the Orbitrap. Database search was performed with Proteome Discoverer 1.0 (Thermo Fisher Scientific software, San José, CA, USA) against Uniprot including fixed modification Carbamidomethylation in Cys and proteome results were statistically analyzed with the Proteome Discoverer software.

After identification, proteins related to the response to biological aging stress lack of fermentable carbon source were selected from both conditions attending to mutant phenotypes, gene ontology terms (GO Terms) or references using YeastMine tool from SGD and Uniprot databases.

To compare quantity of proteins detected in both conditions, a relative quantification has been carried by using the exponentially modified protein abundance index or emPAI (Ishihama *et al.*, 2005): $\text{emPAI} = 10^{\text{PAI}} - 1$. The PAI index is obtained by dividing the observed peptides (taking into account the charge state and missed cleavages) of a specific protein in the analysis by the observable peptides. The observable peptides were determined by using the "MS Digest" software". The protein relative contents in each condition were calculated using the next equation, being Mr the protein molecular weight: Protein content (weight %)= $(\text{emPAI} \times \text{Mr} / \sum (\text{emPAI} \times \text{Mr})) \times 100$.

RESULTS AND DISCUSSION

A total of 413 proteins were identified under BAC and 611 under RFC. These proteins from both conditions were subjected to relative quantification through the EmPAI value identifying proteins more abundant in each condition (Table 1) and at last, only proteins related to the lack of fermentable carbon source were selected according to their phenotypes, GO Terms or references. Proteins average content were: 0,24 in BAC and 0,16 in RFC.

Specifically, 28 proteins were detected under BAC to be related with the lack of fermentable

carbon source while 33 in RFC (Table 2). These values correspond to 6,8% and 5,4%, in BAC and RFC, respectively. From the 28 BAC proteins, 24 were implicated in the biofilm formation biological process and in RFC, 28 out of 33. The rest of proteins (Asc1p, Mtl1p, Pof1p, Ssb1p, Ssb2p and Ste20p) are involved in the response to glucose starvation.

Most of the stress response proteins are localized in cytoplasm and organelles like mitochondria, nucleus and membrane; being the first of them known to play an important role under a condition such as BAC in terms of alternative carbon sources consumption and the formation of biofilm (Moreno-García *et al.*, 2014). 9 lack of fermentable carbon source stress-proteins were detected in the analysis to be localized in the mitochondria: Tuf1p and Idh1p in the mitochondrial matrix; Idh1p, Qcr6p and Por1p in the mitochondrial intermembrane space; Idh1p and Idh2p in the mitochondrial isocitrate dehydrogenase complex, in the mitochondrial membrane, Idh1p and Pdb1p in the mitochondrial nucleoid, mitochondrial membrane part and Pdb1p in mitochondrial pyruvate dehydrogenase complex; while Cpr3p, Ssa2p and Tdh1p are not known to be localized in a mitochondrial part specifically.

Also the majority shows molecular functions as ATP binding, nucleotide binding and cystathionine beta-synthase activity (intermediate step in the synthesis of cysteine) and are involved in biological processes beside those mentioned in the table 2 like oxidation-reduction process, general response to stress, cytoplasmic translation, protein folding, biosynthesis of trehalose which constitutes a major reserve carbohydrate in *Saccharomyces cerevisiae*; and tricarboxylic acid cycle essential for the catabolism of two-carbon molecules together with the glyoxylate cycle and gluconeogenesis.

As it has been observed the number of proteins related to the stress was pretty similar, however proteins under the two conditions were not the same (Table 2 and Fig. 2): 14 proteins were detected under BAC and not under RFC

Tab. 1. Number of proteins detected specifically in the biological aging condition (BAC) and in the reference fermentative condition (RFC), in both conditions, those detected in higher abundance in BAC (BAC > RFC) and in RFC (RFC>BAC) and with similar conditions (BAC $\approx$ RFC).

Type of counting	Nr proteins
BAC specific	206
RFC specific	404
Both conditions	207
BAC > RFC	65
RFC < BAC	45
BAC $\approx$ RFC	97

Tab. 2. Proteins detected that participate in the response to the lack of fermentable carbon source.

Protein	Presence in the condition		Uniprot entry	Protein names	Subcellular location	Biological process
	BAC	RFC				
Asc1p	X	X	P38011	Guanine nucleotide-binding protein subunit beta-like protein (Receptor for activated C kinase) (Receptor of activated protein kinase C 1) (RACK1)	Cytoplasm.	Response to glucose starvation
Mtl1p	X		P53214	Protein MTL1 (MID two-like protein 1)	Membrane; Single-pass type I membrane protein.	
Pof1p		X	P25576	Promoter of filamentation protein 1		
Ssb1p	X	X	P11484	Heat shock protein SSB1 (Cold-inducible protein YG101)	Cytoplasm.	
Ssb2p	X	X	P40150	Heat shock protein SSB2	Cytoplasm.	
Ste20p		X	Q03497	Serine/threonine-protein kinase STE20 (EC 2.7.11.1)	Cytoplasm. Nucleus.	
Arg8p		X	P18544	Acetylornithine aminotransferase, mitochondrial (ACOAT) (EC 2.6.1.11)	Mitochondrion matrix.	Biofilm formation
Asc1p	X	X	P38011	Guanine nucleotide-binding protein subunit beta-like protein (Receptor for activated C kinase) (Receptor of activated protein kinase C 1) (RACK1)	Cytoplasm.	
Atg11p	X	X	Q12527	Autophagy-related protein 11 (Cytoplasm to vacuole targeting protein 9)	Preautophagosomal structure membrane; Peripheral membrane protein. Vacuole membrane; Peripheral membrane protein.	
Ccw14p	X	X	O13547	Covalently-linked cell wall protein 14 (Inner cell wall protein)	Secreted > cell wall. Membrane; Lipid-anchor > GPI-anchor.	
Coq5p		X	P49017	2-methoxy-6-polyprenyl-1,4-benzoquinol methylase, mitochondrial (EC 2.1.1.201) (Ubiquinone biosynthesis methyltransferase COQ5)	Mitochondrion.	
Cox15p		X	P40086	Cytochrome c oxidase assembly protein COX15	Mitochondrion inner membrane; Multi-pass membrane protein.	
Cpr3p	X	X	P25719	Peptidyl-prolyl cis-trans isomerase C, mitochondrial (PPIase C) (EC 5.2.1.8) (Cyclophilin C) (PPI-III) (Rotamase C)	Mitochondrion matrix.	
Cys4p	X		P32582	Cystathionine beta-synthase (EC 4.2.1.22) (Beta-thionase) (Serine sulfhydryase) (Sulfur transfer protein 4)		
Hat2p	X		P39984	Histone acetyltransferase type B subunit 2	Cytoplasm. Nucleus.	
Hcr1p		X	Q05775	Eukaryotic translation initiation factor 3 subunit J (eIF3j) (Eukaryotic translation initiation factor 3 30 kDa subunit) (eIF-3 30 kDa)	Cytoplasm.	
Hsp12p	X	X	P22943	12 kDa heat shock protein (Glucose and lipid-regulated protein)		

Tab. 2. (continued) Proteins detected that participate in the response to the lack of fermentable carbon source.

Protein	Presence in the condition		Uniprot entry	Protein names	Subcellular location	Biological process
	BAC	RFC				
Idh1p	X		P28834	Isocitrate dehydrogenase [NAD] subunit 1, mitochondrial (EC 1.1.1.41) (Isocitric dehydrogenase) (NAD(+)-specific ICDH)	Mitochondrion.	Biofilm formation
Idh2p	X		P28241	Isocitrate dehydrogenase [NAD] subunit 2, mitochondrial (EC 1.1.1.41) (Isocitric dehydrogenase) (NAD(+)-specific ICDH)	Mitochondrion matrix.	
Lys20p	X		P48570	Homocitrate synthase, cytosolic isozyme (EC 2.3.3.14)	Cytoplasm.	
Pdb1p	X	X	P32473	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (EC 1.2.4.1) (Pyruvate dehydrogenase complex component E1 beta) (PDHE1-B)	Mitochondrion matrix.	
Pex1p	X		P24004	Peroxisomal ATPase PEX1 (Peroxin-1) (Peroxisomal assembly protein 1) (Peroxisome biogenesis protein PAS1)	Cytoplasm. Peroxisome membrane; Peripheral membrane protein; Cytoplasmic side.	
Por1p	X		P04840	Mitochondrial outer membrane protein porin 1 (Voltage-dependent anion-selective channel protein 1) (VDAC-1)	Mitochondrion outer membrane.	
Prb1p		X	P09232	Cerevisin (EC 3.4.21.48) (Proteinase YSCB) (Vacuolar protease B) (PrB)	Vacuole.	
Pre9p		X	P23638	Proteasome subunit alpha type-3 (EC 3.4.25.1) (Macropain subunit Y13) (Multicatalytic endopeptidase complex subunit Y13) (Proteasome component Y13) (Proteinase YSCE subunit 13)	Cytoplasm. Nucleus.	
Pst2p	X	X	Q12335	Protoplast secreted protein 2	Secreted.	
Qcr6p	X	X	P00127	Cytochrome b-c1 complex subunit 6 (Complex III subunit 6) (Complex III subunit VI) (Cytochrome c1 non-heme 17 kDa protein) (Mitochondrial hinge protein) (Ubiquinol-cytochrome c reductase complex 17 kDa protein)	Mitochondrion inner membrane.	
Rad23p	X	X	P32628	UV excision repair protein RAD23	Nucleus. Cytoplasm.	
Rad6p	X		P06104	Ubiquitin-conjugating enzyme E2 2 (EC 6.3.2.19) (Radiation sensitivity protein 6) (Ubiquitin carrier protein UBC2) (Ubiquitin-protein ligase UBC2)	Cytoplasm. Nucleus.	
Ras2p		X	P01120	Ras-like protein 2	Cell membrane; Lipid-anchor.	
Rhr2p		X	P41277	Glycerol-1-phosphate phosphohydrolase 1 ((DL)-glycerol-3-phosphatase 1) (EC 3.1.3.21) (Related to HOR2 protein 2)	Cytoplasm. Nucleus.	
Rps7ap	X	X	P26786	40S ribosomal protein S7-A (RP30) (RP40)	Cytoplasm. Nucleus > nucleolus.	

Tab. 2. (continued) Proteins detected that participate in the response to the lack of fermentable carbon source.

Protein	Presence in the condition		Uniprot entry	Protein names	Subcellular location	Biological process
	BAC	RFC				
Rrg7p		X	Q08774	Required for respiratory growth protein 7, mitochondrial	Mitochondrion.	
Sec28p		X	P40509	Coatomer subunit epsilon (Epsilon-coat protein) (Epsilon-COP)	Cytoplasm. Golgi apparatus membrane; Peripheral membrane protein; Cytoplasmic side. Cytoplasmic vesicle > COPI-coated vesicle membrane; Peripheral membrane protein; Cytoplasmic side.	
See1p		X	P40516	N-lysine methyltransferase SEE1 (EC 2.1.1.-) (Secretion and early endocytosis protein 1)	Cytoplasm.	
Ssa2p	X	X	P10592	Heat shock protein SSA2	Cytoplasm. Secreted > cell wall.	
Sti1p		X	P15705	Heat shock protein STI1	Cytoplasm.	
Tdh1p	X	X	P00360	Glyceraldehyde-3-phosphate dehydrogenase 1 (GAPDH 1) (EC 1.2.1.12)	Cytoplasm.	
Tfs1p	X		P14306	Carboxypeptidase Y inhibitor (CPY inhibitor) (CDC25 suppressor 1) (I(C)) (Ic) (Protein DKA1) (Protein NSP1)	Cytoplasm.	Biofilm formation
Tom70p		X	P07213	Mitochondrial import receptor subunit TOM70 (70 kDa mitochondrial outer membrane protein) (Translocase of outer membrane 70 kDa subunit)	Mitochondrion outer membrane; Single-pass membrane protein.	
Tps1p		X	Q00764	Alpha,alpha-trehalose-phosphate synthase [UDP-forming] 56 kDa subunit (EC 2.4.1.15) (General glucose sensor subunit 1) (Glycogen metabolism control protein GLC6) (Trehalose synthase complex catalytic subunit TPS1) (Trehalose-6-phosphate synthase) (UDP-glucose-glucosephosphate glucosyltransferase)	Cytoplasm.	
Tps2p	X		P31688	Trehalose-phosphatase (EC 3.1.3.12) (Trehalose synthase complex catalytic subunit TPS2) (Trehalose-6-phosphate phosphatase) (TPP)	Cytoplasm.	
Tuf1p	X		P02992	Elongation factor Tu, mitochondrial (tufM)	Mitochondrion.	
Ycr051wp		X	P25631	Ankyrin repeat-containing protein YCR051W		
Yfr018cp		X	P43599	Uncharacterized protein YFR018C		
Ygp1p	X		P38616	Protein YGP1 (GP38)	Secreted.	

while 18 were detected under RFC and not under BAC. Proteins detected only in BAC or with more abundance under this condition were: Mtl1p, Ccw14p, Cpr3p, Cys4p, Hat2p, Idh1p, Idh2p, Lys20p, Pex1p, Por1p, Qcr6p, Rad6p, Rps7Ap, Tdh1p, Tfs1p, Tps2p, Tuf1p and Ygp1p.

All of them unless Mtl1p participate in the biofilm formation biological process. Mtl1p was only found in BAC with a protein content of 0,29 (Fig. 2). This protein is a putative plasma membrane sensor involved in cell integrity signaling and stress response during glucose starvation and oxidative stress through TOR1 and RAS2 inhibition (Petkova *et al.*, 2010), whose protein presence were not reported under BAC.

The rest of the BAC proteins besides being involved in the biofilm formation participate also in processes like oxidation-reduction process (Idh1p, Idh2p, Tdh1p and Qcr6p), tricarboxylic acid cycle (Idh1p and Idh2p) or chromatin silencing at telomere (Hat2p and Rad6p). Ccw14p or covalently-linked cell wall protein 14 detected

in a higher content under BAC, up to 2.68-fold higher than under RFC, is a component of the inner layer of the cell wall which at the same time could be found in the extracellular region. This protein is extensively O-glycosylated and its presence might be relevant for the cell wall hydrophobicity which at the same time contributes to the yeast buoyancy and biofilm formation (Zara *et al.*, 2012). Meanwhile, the heat shock protein Hsp12p may play a role in a switch from carbohydrate utilizing metabolism to fatty acid utilizing metabolism at the same time that in cell adhesion (Zara *et al.*, 2002) and it is known to be induced by glucose starvation and by fatty acids (Stone *et al.*, 1990).

Proteins like Ygp1p and Ssa2p were detected under BAC in a very high quantity if related to the relative quantity average which is 0,24 in BAC. The first of them reaching 1,12 and not detected under RFC is an extracellular protein involved in the cellular amino acid metabolic process and in the yeast cell wall assembly. Vandenbosch *et al.* (2013) observed a reduced biofilm formation in YGP1 null mutants. These two facts may shed importance to the this gene in order to do further genetic and phenotypic analyses and further produce yeast strains with enhanced velum formation. The second one, Ssa2p, with a 2,18 protein content and up to 3,01 in RFC, is a cytoplasmic protein or could be found in the cell wall that participates in the response to stress and protein folding biological processes.

CONCLUSION

As a conclusion, it can be said that a similar number of proteins were identified in the flor yeast *Saccharomyces cerevisiae* G1 under a biological aging condition and under reference fermentative condition to be involved in the response to the lack of fermentable carbon source. Most of them participates in the biofilm forming condition. A sensor protein, Mtl1p, involved in stress response during glucose starvation was only detected under the medium lacking a fermentable carbon source and biofilm formation proteins Ygp1p and Ssa2p were found highly expressed under this condition.

This study together with other experiments that definitively confirm the necessity of these proteins synthesis, may lead to the genetic improvement of flor yeast strains aimed to enhance their survival during the biological aging process and hence the whole wine elaboration process.

Fig. 2. Proteins involved in the response to lack of fermentable carbon. Biological aging condition (BAC) protein quantity is represented in red while reference fermentative condition (RFC) is shown in blue.

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Rinsing Device for Separating Seeds of the Species from the *Solanaceae* Botanical Family

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ABSTRACT

In the years 2013-2014, the research was carried out to develop a new rinsing device for separating seeds of the species from the *Solanaceae* botanical family. At first, the design was done, then, the prototype of wood was constructed. The machine was tested on the tomato seeds of the cultivar Frodo F₁ in the Polish seed company breeding tomatoes. The device allowed to increase seed germination in both series of trials. In case on cleaning seeds with the flesh, the seed germination in the best fraction improved from 56.2% (check) to 79.4%. In the case of the seeds only (without flash), the seeds germination improved in the best fraction from 63.2 % (check) to 92 %. In both cases, it was due to lowering to amount of abnormal seedlings. Better quality of the graded seeds was followed by heavier seeds. The device needs further testing on the seeds of other cultivars and species.

Keywords: *seed fractions, seed quality grading, seed rinsing, tomato seed production*

INTRODUCTION

Production of seeds of high quality requires a large amount of work and often specialized equipment (Szpakowska, Hołubowicz 2012). The first step of processing takes place already in the field when the harvesting is done (Copeland, McDonald 1985). The processed seeds should be dry and healthy (Domoradzki, Korpala 2004). Seed cleaning can be divided into 3 stages: primary, basic and individual. The first one includes removing the biggest and heaviest impurities, whereas the basic one uses the same set of machines for different species of seeds. Individual seeds cleaning focuses on selected characters of a given species in order to clean them up to required quality standard, e.g. colour or shape (George 1985; Kelly, George 1998).

Tomato has been the most important vegetable species in the world (FAO 2014). Every year, in the world, there are about 33 million tons of tomato fruits produced, i.e. 10 % of the total area of all vegetable species (Bocian 2006). In 2014, there was an increase in this production to almost 39 million tons (Anonymous 2014). Production of tomato fruits in Poland also increased from 312

(2000) to 760 thousand tons (2012) (FAOstat 2014). This large production needs both good cultivars and seeds. Tomato seeds, before they go on sale, require additional processing and conditioning work (George 1985; Kelly, George 1998). At first, they must be extracted from the fruit pulp. To do so, it is necessary to ferment the pulp with seeds. The pulp with the seeds is then rinsed with water. Seed companies use special machines for it. The whole process consists of three steps: fermentation, the rinsing the pulp with water and centrifugation to remove water and residues (Demir 1994). This method effectively separates the seeds from the pulp but further processes require drying the seeds. Seed companies breeding tomato and producing its seeds have been looking for new devices to minimize the time and cost of production and maximize profits (Hołubowicz, Cieřlik 2005). Such devices will not only lower the costs of tomato seeds production, but should also improve the final seed quality.

The objective of this experiment was to design, build and test a rinsing device for separating seeds of the species from the *Solanaceae* botanical family while improving the quality of seeds.