

## **Changes of properties of wheat flour dough by combination L-ascorbic acid with reducing or oxidising agents**

**Pavína Pečivová<sup>1,3</sup>, Vladimír Pavlínek<sup>2</sup>, Jan Hrabě<sup>1</sup>**

<sup>1</sup> *Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata University in Zlín, T.G.Masaryka 275, 762 72 Zlín, Czech Republic*

<sup>2</sup> *Polymer Centre, Faculty of Technology, Tomas Bata University in Zlín, T.G.Masaryka 275, 762 72 Zlín, Czech Republic*

*xpecivovap@seznam.cz*

### **Quality improvement of wheat flour dough**

#### **Abstract**

Changes of properties of wheat flour doughs with reducing and oxidising agents and their combinations were measured on an oscillatory rheometer. A small addition of L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w) to L-cysteine hydrochloride monohydrate leads to a lower reducing ability of L-cysteine hydrochloride monohydrate and the dough shows higher tenacity and lower extensibility. On the other hand, the same addition of L-ascorbic acid to inactivated dry yeast leads to an increase in the oxidising effect of the dough. L-ascorbic acid (oxidized by oxygen in the dough to dehydroascorbic acid) was found to be the strongest oxidising agent within the agents used while L-threonine was found to be the weakest oxidising agent. The combination of L-ascorbic acid+L-tryptophan had a stronger strengthening influence on the gluten network of wheat flour dough than the combination of L-ascorbic acid+L-threonine. Mainly combinations L-tryptophan and L-threonine with L-ascorbic acid showed interesting and new results.

**Keywords:** amino acid, dough, extensibility, tenacity

#### **Abbreviations:**

AsA L-ascorbic acid

ID inactivated dry yeast

G' the storage modulus

G'' the loss modulus

tan  $\delta$  the loss tangent

$\omega$  angular frequency

## **Introduction**

Nowadays, the quality of wheat flour is very important in the baking industry. This quality is influenced by changes in climatic conditions (e.g. rainfall, temperatures), storage conditions, technology of milling, etc. Wheat flour has either quality gluten (“strong flour”) or damaged gluten (“weak flour”).

The main characteristic of bakery products is their volume and impalpability in the baking industry and the ability to bake perfect bread from flour of different quality. It is very important to use suitable bakery additives which are able to change the qualities of flour and dough to required viscoelastic properties.

Additives influence the gluten network when breaking (reducing agents) or create bonds (oxidising agents) in wheat flour dough and thus change its viscoelastic properties and provide bakery products which have e.g. big volume, impalpable breadcrumb, good porosity, etc.

Wheat gluten forms a three-dimensional network and its properties such as extensibility and elasticity determine properties of wheat flour dough (Kasarda, 1999; Masci et al., 1999; Shewry and Tatham, 1997; Skerritt et al., 1999a, 1999b; Rao et al., 2000; Weegels et al., 1996a, 1996b; Wieser, 2007). Gluten proteins are collectively designated as gliadins and glutenins. Glutenins are polymeric proteins in which the individual subunits are linked by disulphide bonds (Mendichi et al., 2008); they improve elasticity and tenacity (Létang et al., 1999). On the other hand, gliadins are monomeric proteins that consist of single chain polypeptides and contribute to the viscous properties of dough (Field et al., 1983a, 1983b). Both (glutenins and gliadins) create a viscoelastic profile of gluten (Weegels et al., 1997). Gliadins can be taken as plasticizers or dissolvents of glutenins. Rheological behaviour of the mixtures of wheat flour and water is established by the presence of gluten phase (Angioloni and Dalla Rosa, 2007; Létang et al., 1999).

Further constituents of flour are albumins and globulins, which include all cereal enzymes and other proteins that do not influence the properties of dough (Gianibelli et al., 2001).

Additives are added in small quantities to wheat flour and wheat flour dough in order to improve some characteristics of the behaviour, technological processing and final quality of dough. Additives are used according to the properties of raw materials, type of bakery product and technological processes.

Additives are able not only to influence the development of dough but also to improve the qualitative characteristics such as colour, texture, volume, taste and sensory properties of the final bakery products (Indrani and Rao, 2006).

Reducing agents are used for weakening the gluten network of dough in low concentrations. They are able to reduce the resistance of dough to deformation and help with the manufacturing of dough. Therefore they are used especially in „strong flour“ (flour with quality gluten) when it is necessary to produce soft bread, for speedup (mixing, fermentation), for better development of dough and a greater volume of bakery products.

Reducing agents such as L-cysteine hydrochloride monohydrate, L-cysteine p.a., inactivated dry yeast (chemically glutathione) and L-ascorbic acid were used. Cysteine is an amino acid which is used for weakening the gluten network in flour in the baking industry. Its sulfhydryl group is able to react as a reducing agent with disulphide bonds which are in the gluten network in wheat flour dough. Cysteine is most often used as hydrochloride for its better solubility. Cysteine is able to react in low concentrations and is added during mixing (Abd Elkhalifa and El-Tinay, 2002; Angioloni and Dalla Rosa, 2007; Bollaín and Collar, 2004; Collar et al., 2000; Collar and Bollaín, 2004; Indrani and Rao, 2006; Kilborn and Tipples, 1973; Mita and Bohlin, 1983; Srinivasan et al., 2000). Cysteine weakens the gluten network of dough and thus reduces the disulfide bonds between proteins (Angioloni and Dalla Rosa, 2007; Bloksma, 1990).

Inactivated dry yeast (chemically it is glutathione) is a reducing agent which increases the extensibility of dough but less than cysteine. It has to be added in a larger amount. Inactivated dry yeast improves the development of dough, reduces the dehydration of dough and bakery products show a better taste and flavour (Koh et al., 1996; Tilley et al., 2001).

Ascorbic acid is a very popular and widely used flour improver in bread products (Joye et al., 2009). Ascorbic acid, despite being itself a reducing agent, can exert an oxidising effect on the properties of dough after its oxidation by atmospheric oxygen during kneading and maturing. It is now generally accepted that the actual improving compound is its first relatively stable oxidation product - dehydroascorbic acid. The oxidation of ascorbic acid to dehydroascorbic acid takes place quickly and is already completed before the end of the mixing stage (Dong and Hosenev, 1995; Hrušková and Novotná, 2003; Joye et al., 2009). It is able to create bonds and significantly strengthen the gluten network in dough.

Typical concentrations used in bread-making depend on the wheat cultivar, the type and storage time of flour, the bread-making process and the type of bread. Ascorbic acid also improves crust characteristics, crumb structure and colour.

On the other hand, oxidising agents improve the retaining of carbon dioxide in dough. Their influence depends on the molecular weight of protein molecules. Oxidising agents create disulphide bonds between the individual gluten molecules and thus strengthen the gluten network of dough (Indrani and Rao, 2006). They also provide bakery products with a large volume and better porosity of crumb. L-tryptophan and L-threonine were used as oxidising agents.

The aim of this work is to describe the influence of reducing and oxidising agents and their combination with L-ascorbic acid on the viscoelastic properties of wheat flour dough, especially in combination of L-ascorbic acid with amino acids such as L-tryptophan and L-threonine, which are normally present in wheat flour and their further addition improves the nutritional value of the final bakery products. Furthermore, amino acids such as L-tryptophan and L-threonine or their combinations with other amino acids are not normally added to dough in order to change or improve the viscoelastic properties of dough.

## Materials and Methods

### Materials and Dough Preparation

White wheat flour T 530 (moisture 14.3%, gluten in dry matter 36.4%, falling number 339 s) was provided by Penam, Kroměříž, Czech Republic. Other information about flour was found by alveoconsistograph analysis (Chopin – Tripette & Renaud, France) according to the methods ISO 5530\_4 (2002) and ICC No: 171 (2008), respectively. Information can be seen in Table 1.

**Table 1.** Alveograph and consistograph characteristics of investigated untreated flour.

Alveograph characteristic	Value	Consistograph characteristic	Value
P*	95 mmH <sub>2</sub> O	PrMax*	2237 mb*
L*	89 mm	TPrMax*	173 s
P/L*	1.06	Tol*	241 s
W*	287 10E <sup>-4</sup> J	D250*	197 mb
		D450*	742 mb

P\*: tenacity; L\*: extensibility; P/L\*: configuration ratio; W\*: deformation energy; PrMax\*: maximum pressure; TPrMax\*: time to reach maximum pressure; Tol\*: time that pressure is higher than PrMAX minus 20%; D 250\*: the drop in pressure at 250 seconds from PrMax minus 20%; D 450\*: the drop in pressure at 450 seconds from PrMax minus 20%; mb\*: (millibar) – the device is calibrated on values mb

Doughs were prepared from flour, redistilled water (250 g) and salt (NaCl, 9 g). They were mixed for 5.5 minutes in a spiral kneader Bosch Profi Mixx 47 MUM 4770/05 (Robert Bosch Hausgeraete GmbH, Germany). Reducing agents as L-cysteine hydrochloride monohydrate (Ireks GmbH, Eppelborn, Germany) within the concentration range of  $0.6 \cdot 10^{-2}$  -  $6.0 \cdot 10^{-2}$  % w/w, inactivated dry yeast (chemically it is glutathione) from Ireks GmbH, Eppelborn, Germany ( $6.0 \cdot 10^{-2}$  -  $18.0 \cdot 10^{-2}$  % w/w), L-ascorbic acid (Merck KGaA, Darmstadt, Germany) within the concentration range of  $2.0 \cdot 10^{-2}$  -  $18.0 \cdot 10^{-2}$  % w/w ( $6.0 \cdot 10^{-2}$  % w/w of L-ascorbic acid was used in combination with L-cysteine hydrochloride monohydrate, inactivated dry yeast, L-threonine, L-tryptophan); oxidising agents as L-threonine (Merck KGaA, Darmstadt, Germany) within the concentration range of  $1.2 \cdot 10^{-2}$  -  $18.0 \cdot 10^{-2}$  % w/w and L-tryptophan (Merck KGaA, Darmstadt, Germany) within the concentration range of  $1.4 \cdot 10^{-2}$  -  $18.0 \cdot 10^{-2}$  % w/w were added in order to modify dough properties.

Before testing, doughs were stored for 50 minutes in a small tight box at room temperature to allow the stresses developed during mixing to relax.

Additions of additives or their combinations were chosen on the basis of their chemical properties (ability to create or reduces the disulfide bonds between proteins). Additions of additives or their combinations were added to flour. 500 g flour and 9 g salt (NaCl) were used for each analysis.

### **Rheological Measurements**

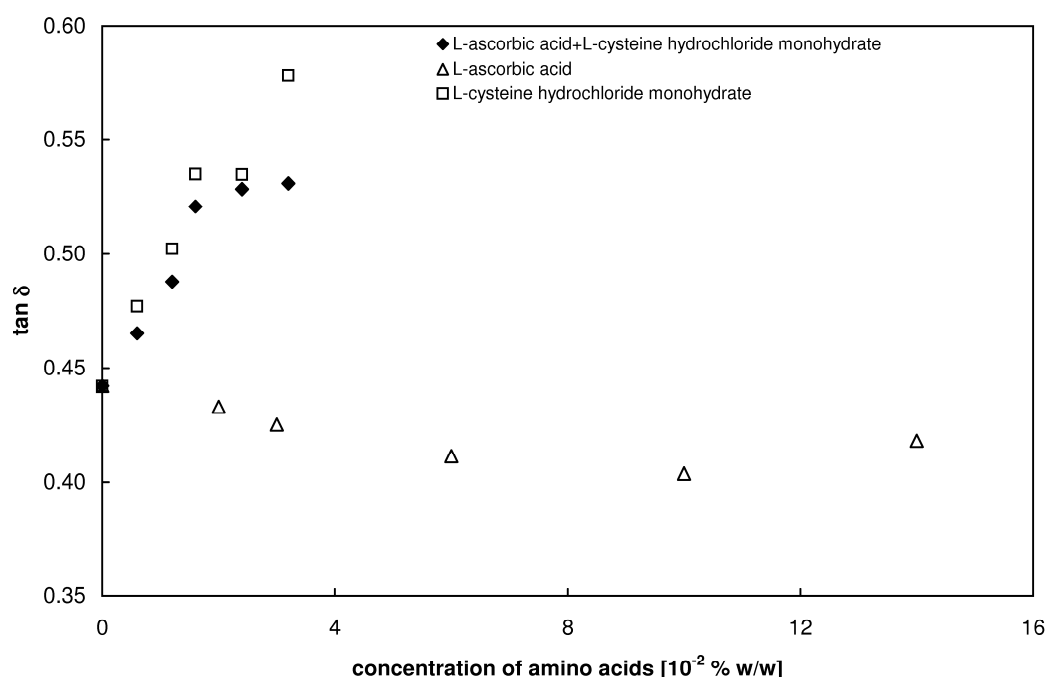
A rotational viscometer Bohlin Gemini (Malvern Instruments, UK) with parallel plate geometry at a temperature of  $(25 \pm 1)$  °C was used. The gap was set at 1.5 mm and the diameter of parallel plates was 25 mm. Before the measurement, the dough sample was left to stand for 5 minutes to allow the induced stresses to relax. The measurements were performed within the area of linear viscoelasticity with a constant stress of 50 Pa. The surface of plates was covered by sandpaper to avoid any slippage problem. The dough edges were cut and coated with silicone oil to prevent moisture loss. Presented results in figures are obtained from 3 repetitions. The storage modulus  $G'$  expressed tenacity and elasticity of wheat flour dough and the loss modulus  $G''$  represented extensibility of wheat flour dough.

### **Results and discussion**

Reducing and oxidising agents or their combinations were used in order to change viscoelastic properties of wheat flour dough.

Reducing agents were used in order to increase the extensibility of dough. On the other hand, oxidising agents were used to increase the elasticity and tenacity of dough.

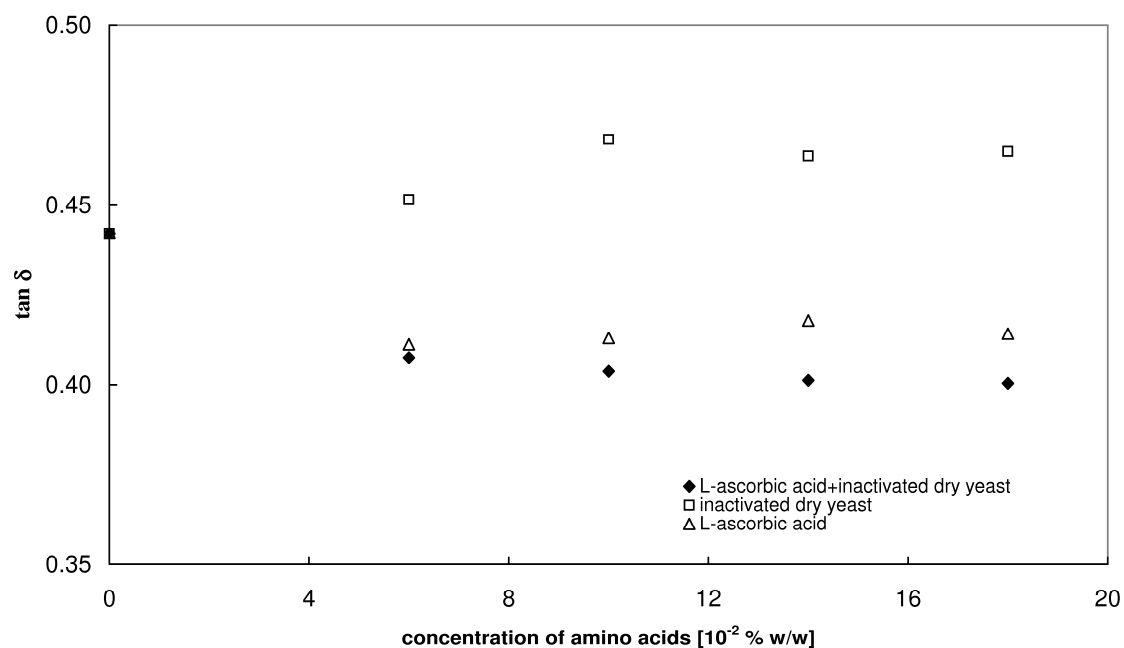
Different effects of reducing and oxidising agents on the viscoelastic properties of dough can be seen in Figure 1. Figure 1 shows the oxidising effect of L-ascorbic acid (the effect of dehydroascorbic acid) as an oxidising agent, the reducing influence of L-cysteine hydrochloride monohydrate and the effect of their combination on dough. A small concentration of L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w) was added to the individual concentrations of L-cysteine hydrochloride monohydrate ( $0.6 \cdot 10^{-2}$  % w/w,  $1.2 \cdot 10^{-2}$  % w/w,  $1.6 \cdot 10^{-2}$  % w/w,  $2.4 \cdot 10^{-2}$  % w/w,  $3.2 \cdot 10^{-2}$  % w/w). It can be seen that the reaction of both agents caused a decrease in the reducing influence of L-cysteine hydrochloride monohydrate in the presence of L-ascorbic acid. The dough becomes more elastic than that with the addition of L-cysteine hydrochloride monohydrate alone. It means that the loss tangent of the combination of L-ascorbic acid+L-cysteine hydrochloride monohydrate decreased.



**Fig. 1.** The influence of L-ascorbic acid+L-cysteine hydrochloride monohydrate on viscoelastic properties of wheat flour dough;  $\square$  loss tangent of L-cysteine hydrochloride monohydrate,  $\Delta$  loss tangent of L-ascorbic acid,  $\blacklozenge$  loss tangent of the combination L-ascorbic acid+L-cysteine hydrochloride monohydrate.

Figure 2 shows the effect of L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w) (the effect of dehydroascorbic acid) as an oxidising agent added to the individual concentrations of a reducing agent ( $6.0 \cdot 10^{-2}$  % w/w,  $10.0 \cdot 10^{-2}$  % w/w,  $14.0 \cdot 10^{-2}$  % w/w,  $18.0 \cdot 10^{-2}$  % w/w), i.e.

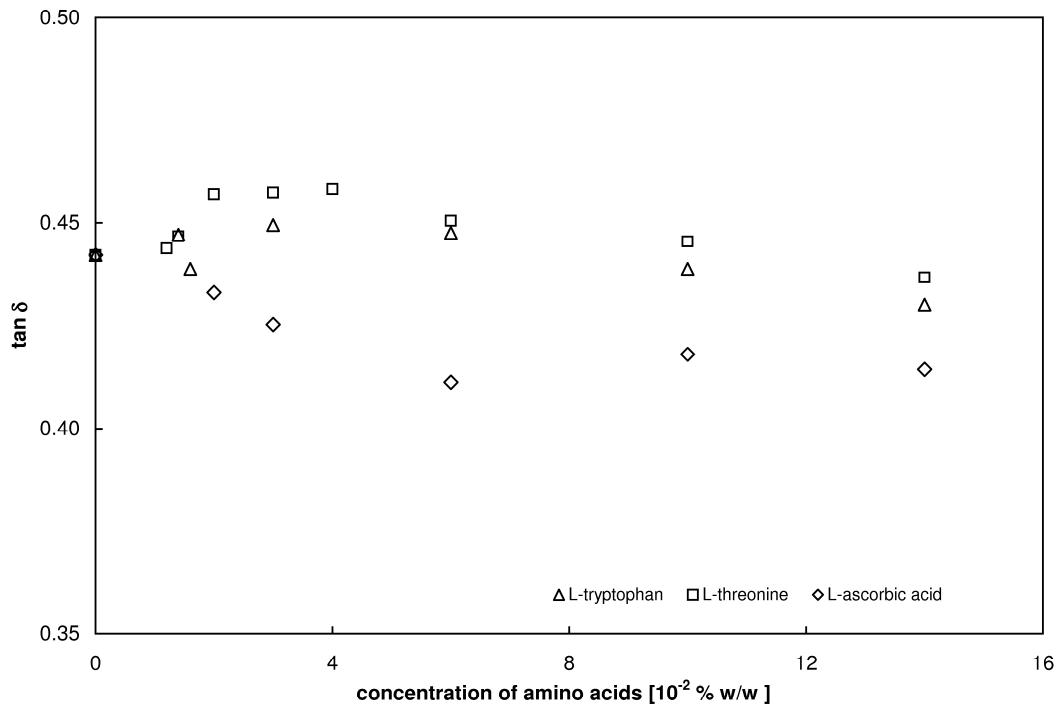
inactivated dry yeast. It can be seen that the reaction between inactivated dry yeast and L-ascorbic acid caused an oxidising effect in the dough. The loss tangent which describes the influence of the combination of L-ascorbic acid+inactivated dry yeast decreased more than the loss tangent of L-ascorbic acid. We suppose that it was caused by the influence of glutamic acid which is one of the main amino acids present in inactivated dry yeast. It is able to react with L-ascorbic acid, creates bonds and has an oxidising effect on dough.



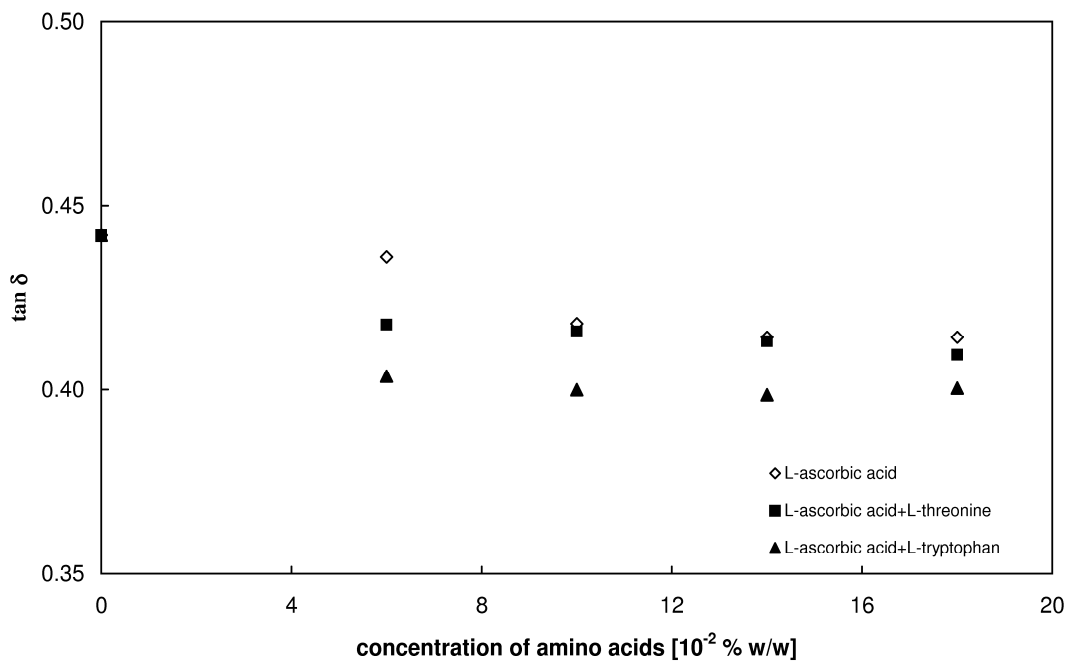
**Fig. 2.** The influence of L-ascorbic acid + inactivated dry yeast on viscoelastic properties of wheat flour dough;  $\square$  loss tangent of inactivated dry yeast,  $\triangle$  loss tangent of L-ascorbic acid,  $\blacklozenge$  loss tangent of the combination L-ascorbic acid+inactivated dry yeast.

Figure 3 describes the effect of oxidising agents on the dough. It can be seen that L-ascorbic acid (the effect of dehydroascorbic acid) was the strongest oxidising agent because the loss tangent showed the lowest values. On the other hand, L-threonine was the weakest oxidising agent and the loss tangent which shows its influence on the dough increased the most.

The effect of oxidising agents can be seen in Figure 4. It was found that the oxidising influence of agents such as L-threonine and L-tryptophan, which were subsequently added at higher concentrations ( $6.0 \cdot 10^{-2}$  % w/w,  $10.0 \cdot 10^{-2}$  % w/w,  $14.0 \cdot 10^{-2}$  % w/w,  $18.0 \cdot 10^{-2}$  % w/w) to the same (small) concentration of L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w), increased. The combination of L-ascorbic acid+L-tryptophan caused the strongest oxidising effect on the dough showing the lowest values of the loss tangent.



**Fig. 3.** The influence of oxidising agents on viscoelastic properties of wheat flour dough; □ loss tangent of L-threonine; △ loss tangent of L-tryptophan; ◇ loss tangent of L-ascorbic acid.



**Fig. 4.** The influence of combination oxidising agents on viscoelastic properties of wheat flour dough; ◇ loss tangent of L-ascorbic acid, ■ loss tangent of the combination of L-ascorbic acid+L-threonine, ▲ loss tangent of the combination of L-ascorbic acid+L-tryptophan.



## **Conclusion**

The results obtained from dynamic oscillatory rheometry confirmed that the individual additives and their combinations influence the viscoelastic properties of wheat flour dough.

It was found that the addition of a low concentration of L-ascorbic acid ( $0.6 \cdot 10^{-2}$  % w/w) to increasing concentrations of L-cysteine hydrochloride monohydrate ( $0.6 \cdot 10^{-2}$  –  $3.2 \cdot 10^{-2}$  % w/w) caused a decrease in the reducing effect of L-cysteine hydrochloride monohydrate. The dough was more elastic and thus less extensible than that with the addition of L-cysteine hydrochloride monohydrate alone.

On the other hand, the reaction between inactivated dry yeast ( $6.0 \cdot 10^{-2}$  –  $18.0 \cdot 10^{-2}$  % w/w) and L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w) caused an increase in the oxidising effect on the dough.

L-ascorbic acid (the effect of dehydroascorbic acid) appeared to be the strongest oxidising agent and L-threonine the weakest oxidising agent among the chemicals investigated. However, a small addition of L-ascorbic acid ( $6.0 \cdot 10^{-2}$  % w/w) to the increasing concentrations of L-threonine and L-tryptophan ( $6.0 \cdot 10^{-2}$  –  $18.0 \cdot 10^{-2}$  % w/w) caused an increase in the oxidising effect on the dough. The combination of L-ascorbic acid+L-tryptophan had the strongest oxidising influence on the dough.

As the above-mentioned results show, the use of either reducing or oxidising agents is not the only way of modifying the properties of dough. Their combination resulting in a synergic effect can also be beneficial (improve quality of final bakery products).

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